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INFECTION OF PERENNIAL DELPHINIUMS BY CALIFORNIA-ASTER-YELLOWS VIRUS[1]

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INTRODUCTION

SEVERAL OBSCURE diseases attack garden varieties of perennial delphiniums (hybrids and horticultural varieties of several species of *Delphinium*) and cause losses to seed companies, nurserymen, and growers. One of these diseases is particularly troublesome, sometimes causing delphiniums grown from seed to fail totally the second year. Some of the choicest seeds, requiring hand-pollination and bagging, are grown in California; and often these hybrid seeds, also selected for mildew resistance, are lost owing to this disease. Because the symptoms resemble those of California aster yellows on other host plants, an investigation was undertaken to determine whether this disease is caused by the virus of California aster yellows.

In the course of the work on this disease, several other delphinium virus diseases and troubles resembling viroses were investigated. The reports of these investigations have been divided into six papers. The present paper is confined to the work with California aster yellows on perennial delphiniums. Experiments with two other naturally occurring viroses of perennial delphiniums are reported in the other papers of this issue (*18, 22*).[3] The attempts to infect perennial delphiniums experimentally with other viruses are reported in a fourth paper (*20*). Two leaf variegations of perennial delphiniums resembling viroses but not infectious were encountered; these are reported in a fifth paper (*19*). Several of the virus diseases attacking perennial delphiniums affect also annual delphiniums, or larkspur (*Delphinium Ajacis*); the experiments with this host plant are published in a sixth paper (*21*).

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[3] Italic figures in parentheses refer to "Literature Cited" at the end of this paper.

California aster yellows is a serious disease of aster and celery, and affects also lettuce (*13*), carrots, parsley, and parsnip (*14*), and many ornamentals (*23*). Except in a preliminary note (25) based on this investigation, however, it has not previously been reported on delphinium. Nevertheless, the symptoms described by several investigators in other states indicate that they may have been dealing with this disease.

In 1927 Linford (*33, 34*) reported 50 per cent of yellows (cause undetermined) on tall perennial delphinium at Logan, Utah; he evidently suspected the disease to be aster yellows but stated that delphiniums were not known to be susceptible to that disease, and conducted no tests.

In 1933 Hungerford (*35*) reported a new virus disease on delphinium at Moscow and Boise Valley, Idaho, and suggested "witches'-broom" as a common name.

Orton (*10*) reported a new virus disease of delphiniums in the Northwest called "greens," which stunts the plants and makes them produce nothing but dwarfed green flowers.

Heald and Burnett (*6*) described a virus disease of perennial delphinium in the State of Washington and applied the name "stunt" to the disease.

A more complete discussion of the same delphinium disease in that state was published by Burnett (*2*), again under the designation "stunt." Several references on the distribution of "stunt" (virus) of delphinium in the states of Washington and New York have appeared in the *Plant Disease Reporter* (*36, 37, 38*).

In the present investigation, the symptoms of the disease on naturally infected delphiniums were compared with those on delphiniums experimentally infected with California aster yellows. Field investigations were undertaken to determine the most important vectors of the virus of delphiniums. Attempts were made to recover the virus from naturally infected delphiniums by the mountain leafhopper, *Thamnotettix montanus* Van D.; the geminate leafhopper, *T. geminatus* Van D.; the short-winged aster leafhopper, *Macrosteles divisus* (Uhl.) (=*Cicadula divisa*), and the long-winged aster leafhopper, a physiological race or variety of the same species (*17*); and to transfer it to healthy aster and celery plants. Attempts were made to infect healthy delphiniums grown from seeds with the virus obtained from diseased aster and celery plants by the four vectors and to recover the virus from the infected delphiniums and transfer it back to healthy aster and celery plants by previously noninfective leafhoppers. The delphinium varieties and hybrids experimentally infected, the incubation period of the disease in delphiniums, and the weed reservoirs of the virus in and near delphinum fields were also investigated.

METHODS

Rhubarb (*Rheum Rhaponticum*) is immune to California aster yellows and was found to be a favorable breeding plant of the geminate leafhopper. Adults collected on delphiniums in the field were confined in cages enclosing rhubarb plants in which they oviposited. The adults were removed from the cages before the nymphs hatched from the eggs. The males reared during the nymphal stages on rhubarb plants, as well as adults of the later generations, were frequently transferred to celery plants, but caused no infection. Males were used instead of females, to avoid egg deposition.

The production of noninfective short-winged aster leafhoppers (reared on mildew-resistant Sacramento barley immune to aster yellows), mountain leafhoppers, and geminate leafhoppers (the last two reared on healthy celery) have been described in previous papers (*14, 16*). The long-winged aster leafhopper also remained noninfective when reared on Sacramento barley.

SYMPTOMATOLOGY

The description of the symptoms of aster yellows pertaining to the abnormal development of the flower is in general terminology, since no anatomical comparison has been made of the normal and abnormal flower structures.

One of the most conspicuous symptoms of aster yellows on naturally infected delphiniums, when roguing of diseased plants is not practiced, is the dwarfing of some of the plants. A general yellowing of the foliage occurred on the stunted plants. It was not uncommon to find a dense cluster of yellow shoots about 6 inches high which never developed spikes. A closer examination of one of these shoots revealed numerous lateral or axillary shoots (plate 1, *A*) bearing abnormal leaves—instead of 3 lobes or divisions, 2 lobes may be dwarfed, or 1 or 2 lobes may be absent (plate 1, *C*), or the blades may be linear or even seem to be reduced to the midvein with threadlike petioles (plate 1, *A*).

Frequently many slender shoots from 6 to 12 inches or more in height grow from the crown. The petioles are elongated with chlorotic leaves, which may be cupped inward (plate 2, *C, D*). These slender shoots may remain dwarfed until the blossoming period or they may develop slender spikes. It was not unusual to find delphinium plants 3 to 5 feet high with spikes bearing abnormal flowers and with slender, chlorotic shoots, which never developed spikes, at the base of the plant.

Other striking symptoms of aster yellows on naturally and experimentally infected delphiniums were several abnormalities in the de-

velopment of the flowers. Green flower buds (plate 3, *A*) expanded into enlarged green leafy sepals and dwarfed petals (plate 3, *B;* plate 4, *A, B*). The most remarkable peculiarity was the appearance in the normal position of the carpels of structures resembling leaves with green blades and petioles; the petals were dwarfed and surrounded by enlarged green sepals (plate 3, *C;* plate 4, *D, F*). Frequently the sepals, petals, carpels, and stamens were replaced by green leafy structures (plate 3, *D*), and sometimes enormous clusters of these abnormal floral parts developed at the apical region of the spike (plate 2, *B*).

Phyllody, or the transformation of stamens, petals, carpels, or all of the floral parts into leafy structures, is frequently caused by parasites, but other disturbances, such as general effects of soil excesses or over-nutrition, may produce similar effects (*5*). Phyllody and virescense, or greening of the flowers, are common symptoms of aster yellows among ornamentals, economic plants, and weeds. In all probability these symptoms described by plant teratologists on many plants are caused by this virus.

A sectorial infection was observed on some delphinium plants in which normal-colored flowers and abnormal flowers with green, leafy sepals and petals occurred on opposite sides of the same spike (plate 2, *A*). Sometimes abnormal flowers with green floral parts were found on the spikes of one or more stalks and apparently normal flowers on the spikes of the remaining stalks. A cluster of apparently normal flowers was present on the apical region and often near the basal region of spikes, with the intermediate or basal region of the spikes bearing filamentous structures (plate 5, *A, B*). Dwarfed flowers with elongated pedicels were found on some spikes (plate 5, *C*). A closer examination of the dwarfed flowers showed median-green areas on the petals (plate 5, *G–J*).

Spikes were observed in the field with clusters of abnormal flowers surrounded by dwarfed single-lobed bracts (plate 5 *D*) or with 3-lobed, dwarfed leaves arranged to form a rosette, with central, dwarfed, floral parts (plate 5, *E, F;* plate 4, *C*). A considerable amount of variation occurred in the formation of the rosette; often it was composed of 3-lobed leaves, dwarfed sepals, and petals (plate 5, *K*) or cupped leaves and sepals, and dwarfed petals (plate 5, *L*). An examination of the central floral parts of some rosettes under the binocular microscope showed peculiar structures which resembled dwarfed flower buds (plate 4, *E*), sometimes one bud attached by a stalk to another bud (plate 4, *G*), or the carpels replaced by a stem bearing variously modified appendages.

Frequently a proliferation of the apical end of the spike occurred and resulted in variable types of malformations, which confused some grow-

ers, who doubted that all of the extremely varied symptoms were produced by a single virus. Sometimes a dense cluster of leaves enclosed abnormal, green flowers (plate 6, *A*); or a cluster of leafy structures replacing normal sepals, petals, carpels, and stamens, with lower normal-shaped leaves (plate 6, *B*); or linear, leafy structures representing abnormal floral parts with lower single-lobed leaves (plate 6, *C*); or a bunchy, tangled mass of flower parts (plate 6, *D*); or an apical, central cluster of abnormal flowers with filamentous flower organs and lateral branches with dwarfed, apical leaves surrounding abnormal green flowers (plate 7, *A*). The abnormal, green flowers with filamentous sepals and petals were not always found in clusters. They often were arranged and spaced on the spikes like those of healthy plants (plate 7, *B*). Sometimes a cluster of green flowers with long pedicels appeared on the apical end of the spike, with lateral branches surrounded by linear leaves (plate 7, *C*). Numerous slender branches of the spike with dwarfed, abnormal, green flowers suggested a witches'-broom appearance (plate 7, *D*).

Delphiniums naturally infected with aster yellows sometimes show necrotic symptoms on the stems, petioles, and blades, but these symptoms are probably caused by secondary bacterial infections and not directly by the aster-yellows virus. Sometimes such dark-brown or black lesions or necrotic streaks of variable size, shape, diameter, and length occurred on the stems (plate 8 *A, D, E*). Sometimes black blotches girdled the stem (plate 8, *G*) and caused death of the shoots (plate 8, *H*). Sometimes cracks in the stems followed the necrotic streaks (plate 8, *F*). The necrotic streaks appeared on the petioles (plate 8, *A*), sometimes at or near their attachment (plate 8, *C*). Black necrotic specks resembling bacterial leaf spot (plate 8, *C*) occurred on the blades; these often coalesced to form large irregular areas (plate 8, *B*), and sometimes spread over and killed the entire leaf (plate 8, *H*). These symptoms were common on delphiniums grown under natural conditions during the second or later years, but have not been observed on seedlings in seedbeds or cold frames or on experimentally infected plants grown in the greenhouse.

Stalks of delphiniums infected with aster yellows may fall over between the rows before or during the blossoming period. Many of the stalks may be severed but others still adhere to the roots, the leaves often wilting (plate 6, *E*). The stalks frequently showed necrotic streaks and often black lesions. In the spring of 1939 few stalks had dropped up to April 18, but by May 3 it was difficult to walk between the rows owing to the number of stalks which had dropped.

Similar necrotic symptoms on the blades, petioles, and stems, and the lodging or falling over of the shoots have been described by Heald and

Burnett (*6*), Burnett (*2*), and Mulford (*9*) with delphinium "stunt" of Washington, but again these symptoms are probably produced by secondary bacterial invaders and not directly by the aster-yellow virus.

Delphinium seedlings experimentally infected with aster yellows developed greatly elongated, vertical or upright, frequently curved, yellow petioles (plate 1, *B*) with dwarfed, chlorotic leafblades often cupped inward. Naturally infected delphinium seedlings showed similar symptoms except that the petioles were shorter (plate 1, *D*). The greatly elongated petioles developed only when kept in cages in the greenhouse where the light intensity is lower. Delphiniums experimentally infected before or after the spikes began to grow during the second season, developed chlorotic, cupped leaves, often with curved petioles (plate 2, *D*).

The effect of the disease on the flowers was noted on experimentally infected varieties or hybrids. Seedlings infected during the spring often produced a bloom late the first year in the glasshouse, and all infected plants produced abnormal green flowers on the dwarfed spikes. Some of the infected seedlings died before spikes began to grow. Delphiniums infected during the second year before the spikes appeared developed abnormal, green flowers with enlarged, cupped sepals, and sometimes leafy structures from the carpels (plate 3, *C;* plate 4, *F*). Delphiniums infected after the spikes appeared the second year sometimes failed to produce green flowers with abnormal floral parts but developed a dense cluster of short, yellow shoots from the crown after blossoming.

INSECT VECTORS

Three species of leafhoppers have previously been reported (*16*) to transmit the California-aster-yellows virus to aster and celery plants. The short-winged aster leafhopper transmitted the virus with greater efficiency than the mountain leafhopper and the geminate leafhopper. The transmission of the virus by the mountain leafhopper to celery averaged 26.1 and to aster 2.9 per cent, and by the geminate leafhopper to celery 13.7 per cent; but this species failed to transmit the virus to asters. In experiments not previously reported, 120 healthy asters were inoculated, using lots of 3 to 30 male or female geminate leafhoppers to each aster, but not a single case of aster yellows developed, even though a total of 697 adults were used, all of which had completed the nymphal stages on diseased celery plants. During 1941, 25 lots of 50 male geminate leafhoppers reared on diseased celery were transferred to healthy celery plants; and after celery yellows developed, each lot was transferred to 2 successive healthy asters. Typical symptoms of aster yellows appeared on 4 of 50 asters inoculated.

Garden varieties of perennial delphiniums are unfavorable food plants of short-winged and long-winged aster leafhoppers. A high mortality occurred on delphiniums; some of the leafhoppers died within 4 to 6 hours and most of them within 24 hours.

Perennial delphiniums are, however, favorable food and breeding plants of the mountain and geminate leafhoppers under natural conditions. Large populations of nymphs and adults of the mountain leafhopper were taken on perennial delphiniums near Mt. Eden and Capitola during the autumn. Nymphs and adults of the geminate leafhopper were abundant on perennial delphiniums near Salinas. The life histories of both species of leafhopper were completed on delphiniums in the greenhouse.

Vectors Collected on Naturally Infected Delphiniums.—Thirteen lots of 20 male or female mountain leafhoppers collected on delphinium naturally infected with aster yellows near Mt. Eden on October 2, 1937, were transferred to healthy Blackmore and Langdon Giant or Summer Cloud delphiniums, one lot to a plant; 11 of 13 plants, or 84.6 per cent, developed typical symptoms of the disease.

A similar test was made with the geminate leafhopper collected on diseased delphiniums near Salinas on September 15, 1937; 12 of 13 plants, or 92.3 per cent, developed symptoms of aster yellows. Four lots of 20 males, which transmitted the virus to the first set of delphiniums, were transferred to a second set of plants, and all of these became diseased. One lot of 20 males failed to transmit the virus to either of 2 successive delphiniums.

Recovery of Virus from Naturally Infected Delphiniums.—A comparison was made of the ability of the four vectors to recover the virus from naturally infected perennial delphiniums obtained from Mt. Eden and to transfer it to healthy aster or celery plants. The mountain leafhopper and short-winged and long-winged aster leafhoppers were tested by feeding each vector on separate infected delphiniums. In another test, all three of these and also the geminate leafhopper were fed on the same infected plant. Previously noninfective males were used in all tests. The number of delphiniums used in each test, the number of insects on each plant, and the length of time the insects were fed on infected delphiniums, and on healthy aster or celery plants when symptoms failed to develop are indicated in table 1. In spite of the short exposure of delphiniums to the aster leafhopper (6 to 18 hours) high mortality reduced the average number of short-winged aster leafhoppers transferred to aster to 16 per plant and that of the long-winged to 17 per plant even at room temperature. Table 1 shows the recovery and transfer of the virus by the four vectors.

Repeated attempts have been made during the past ten years to recover the virus from naturally infected delphiniums by means of the short-winged aster leafhopper, but all efforts failed owing to the fact that this insect rarely picks up the virus in short feeding periods from diseased delphiniums.

TABLE 1

Recovery of California-Aster-Yellows Virus from Naturally Infected Delphiniums by Four Vectors

Vector	Length of time fed		Delphiniums naturally infected	Insects on each delphinium	Plants inoculated		Plants infected		Percentage infected
	On delphinium	On aster or celery			Aster	Celery	Aster	Celery	
	days	*days*	*number*	*number*	*number*	*number*	*number*	*number*	*per cent*
Mountain leafhopper (*Thamnotettix montanus*)	7	32	9	20	0	9	..	0	0.0
Short-winged aster leafhopper (*Macrosteles divisus*)	¼–¾	39	25	25–30	25	0	1	..	4.0
Long-winged aster leafhopper (*M. divisus*)	¼–¾	36	19	25–30	19	0	4	..	21.0
Mountain leafhopper (*T. montanus*)*	7	32	24	20	0	24	..	5	20.8
Geminate leafhopper (*T. geminatus*)*	6	34		20	0	24	..	1	4.2
Short-winged aster leafhopper (*M. divisus*)*	¼–¾	39		25–30	24	0	0	..	0.0
Long-winged aster leafhopper (*M. divisus*)*	¼–¾	36		25–30	24	0	4	..	16.7
All vectors			77	.....	92	57	9	6	10.1

* All four vectors were fed in succession on an infected plant and then transferred separately to healthy aster or celery plants.

EXPERIMENTAL INFECTION

Delphinium Varieties and Hybrids.—The following delphinium varieties and hybrids[4] grown from seeds were experimentally infected with the aster-yellows virus by means of four vectors. No attempt has been

[4] Of these delphiniums grown from seeds, nos. 1, 2, and 3 are from Ferry-Morse Seed Co., San Francisco; no. 4 from Germain Seed and Plant Co., Los Angeles; nos. 5, 6, 7, 8, 9, 10, and 11 from Hallawell Seed Co., San Francisco; no. 12 from Aggeler & Musser Seed Co., Los Angeles; and nos. 13 and 14 from Henry A. Dreer, Philadelphia, Pa.

made to determine the species in most cases, since the origin of many garden varieties, hybrids, and races of delphiniums is unknown.

1. Blackmore and Langdon Giants
2. Dwarfed Chinese Blue Butterfly
3. Tall hybrid Bellamosum
4. Pacific Giant strain
5. Cambridge Blue No. 2828
6. Chinese Azure Blue No. 2821
7. Chinese Dark Blue No. 2822
8. Chinese Blue Butterfly No. 2823
9. Cliveden Beauty No. 2837
10. *Delphinium grandiflorum* var. *album*
11. Improved Wrexham or Hollyhock strain
12. A & M Sunbeam hybrids
13. Dreer's De Luxe hybrids No. 2160
14. White Elatum (Summer Cloud) No. 2149

Incubation Period of the Disease.—The incubation period of the disease was determined in delphinium seedlings and during the second year's growth before and after spikes developed, the later tests being conducted to ascertain the effect of the disease on the flower parts.

Four delphinium varieties or hybrids grown from seeds were inoculated during June. The 12 to 15 plants of each kind were divided among the four vectors, each plant being inoculated singly by one lot of 20 males of one vector. Short-winged and long-winged aster leafhoppers all died within 1 day. The cages containing the mountain or geminate leafhoppers were removed at the end of 1 day from the seedlings and replaced with empty cages. In this experiment all delphinium seedlings inoculated by any of the four vectors developed symptoms of the disease. The length of time from the day of inoculation until the petioles became chlorotic, elongated, vertical, with dwarfed, chlorotic, frequently cupped leaves, was considered as the incubation period of the disease. The results obtained are indicated in table 2. The check, or control, plants of all delphinium varieties and hybrids remained healthy.

The incubation period of the disease was also determined in Blackmore and Langdon Giants and Wrexham delphiniums grown from seeds, kept in a glasshouse during the summer and most of the winter, and inoculated during February, March, and April. To determine the effect of the disease on the flower parts, some of the delphiniums were inoculated before the spike developed and others after the spike began to appear. Each delphinium was exposed to one lot of 20 infective males, one species of leafhoppers being used on each plant. The number of

TABLE 2

INCUBATION PERIOD OF CALIFORNIA ASTER YELLOWS IN DELPHINIUM SEEDLINGS INFECTED DURING JUNE BY FOUR VECTORS*

Variety of delphinium	Plants infected	Incubation period of disease	
		Range	Mean
	number	*days*	*days*
Blackmore and Langdon Giant	12	15–43	27.0
Chinese Azure Blue	12	15–17	16.3
Chinese Dark Blue	15	15–43	17.4
Chinese Blue Butterfly	14	15–43	19.6
All varieties	53	.....	19.5

* Each kind of delphinium was tested with all four vectors (mountain leafhopper, *Thamnotettix montanus;* geminate leafhopper, *T. geminatus;* and short-winged and long-winged aster leafhoppers, *Macrosteles divisus*), each plant being inoculated singly by one lot of 20 males of one vector.

TABLE 3

INCUBATION PERIOD OF CALIFORNIA-ASTER-YELLOWS DISEASE IN DELPHINIUMS* INOCULATED DURING FEBRUARY TO APRIL OF THE SECOND YEAR'S GROWTH

Vector	Plants inoculated	Plants infected	Plants healthy	Incubation period of disease	
				Range	Mean
Delphiniums inoculated before spikes developed					
	number	*number*	*number*	*days*	*days*
Mountain leafhopper (*Thamnotettix montanus*)	6	6	0	29–51	43.7
Geminate leafhopper (*T. geminatus*)	5	5	0	27–81	59.0
Short-winged aster leafhopper (*Macrosteles divisus*)	11	7	4	19–81	45.9
Long-winged aster leafhopper (*M. divisus*)	7	7	0	22–43	30.1
All vectors	29	25	4	.....	43.5
Delphiniums inoculated after spikes developed					
	number	*number*	*number*	*days*	*days*
Mountain leafhopper (*T. montanus*)	5	5	0	29–66	45.8
Geminate leafhopper (*T. geminatus*)	5	4	1	28–44	32.0
Short-winged aster leafhopper (*M. divisus*)	5	0	5	.....	....
Long-winged aster leafhopper (*M. divisus*)	5	5	0	28–106	55.6
All vectors	20	14	6	.....	45.0

* Blackmore and Langdon Giants and Wrexham.

days from the inoculation of the plant until the youngest leaves at the crown of the plant became chlorotic or until green abnormal flower parts developed was considered as the incubation period of the disease; this is shown in table 3. The check plants of both varieties remained healthy.

A comparison of the results obtained in tables 2 and 3 shows that the average incubation periods of the disease in delphinium seedlings infected during June of the first season's growth are shorter than in delphiniums inoculated during February, March, and April during the second year's growth. Temperature and thrifty or vigorous growth probably play important roles in the duration of the incubation period of the disease.

TABLE 4

SUMMARY OF RECOVERY OF CALIFORNIA-ASTER-YELLOWS VIRUS BY EACH VECTOR FROM NATURALLY AND EXPERIMENTALLY INFECTED DELPHINIUMS WHEN TRANSFERRED TO HEALTHY ASTER OR CELERY PLANTS

Vector	Delphiniums tested	Delphiniums from which virus was recovered	Percentage recovery of virus			
			From naturally infected delphiniums (table 1)	From experimentally infected delphiniums	From delphiniums in which incubation period was determined (tables 2, 3)	Weighted mean
	number	*number*	*per cent*	*per cent*	*per cent*	*per cent*
Mountain leafhopper (*Thamnotettix montanus*)	71	13	15.1	18.5	27.3	18.3
Geminate leafhopper (*T. geminatus*)	56	8	4.1	15.0	33.3	14.3
Short-winged aster leafhopper (*Macrosteles divisus*)	104	4	2.2	9.4	0.0	3.8
Long-winged aster leafhopper (*M. divisus*)	94	11	18.6	7.1	11.6	11.7

The infection of delphiniums by four vectors during the second year's growth before spikes appeared was 86.2 per cent and after spikes developed was 70.0 per cent.

Recovery of Virus from Experimentally Infected Delphiniums.—The virus was recovered from experimentally infected delphinium varieties or hybrids, including those plants in which the incubation period of disease was determined. The percentages of recovery of the virus by each vector from naturally and experimentally infected delphinium are shown in table 4, together with the number of recoveries of the virus with each vector and the total number of trials from which the average percentages were computed.

RESERVOIRS OF VIRUS

It is evident that perennial delphiniums serve as reservoirs of the aster-yellows virus. Some commercial growers practice roguing of diseased delphiniums from their fields but pay no attention to the weed reservoirs of the virus. Prickly sow thistle (*Sonchus asper*) growing among delphiniums and along the margin of the fields near Capitola

was commonly found to be naturally infected with aster yellows. Cheeseweed (*Malva parviflora*) was demonstrated to be naturally infected with the disease near Mt. Eden. A large number of weeds have been experimentally infected and many weeds have been demonstrated to be naturally infected with the virus.

CONTROL

Commercial delphinium growers plant seeds in seedbeds or cold frames. An examination of delphinium seedlings grown in seedbeds near Hayward showed the presence of the mountain leafhopper on December 22, 1938, but the geminate leafhopper was not collected; the latter may winter over in the egg stage. Aster yellows and other virus diseases of delphinium were common in cold frames and increased until they were transplanted in the field. Each virus-diseased delphinium transplanted in the field is a source of spread to healthy plants by insects. One grower raised delphinium seedlings in the cold frames under muslin covers to keep out leafhoppers and aphids. Among these seedlings, examined at intervals of 2 weeks until they were transplanted in the field, not a single virus-diseased plant was found.

One commercial grower practiced roguing of diseased delphiniums, and his fields were kept free from weeds, but delphinium aster yellows continued to appear. The mountain leafhopper was extremely abundant in delphinium fields during the summer and autumn in this district.

The weeds on which the four vectors of the aster-yellows virus complete their life histories under greenhouse conditions are being investigated. Field investigations must be undertaken to find the breeding plants of the mountain and geminate leafhoppers and to learn when the flights into the delphinium fields occur, before a spray program can be undertaken. One commercial grower began spraying operations during the spring, but not a single vector of the virus was captured by sweeping delphiniums with an insect net during that season.

The failure of delphiniums grown in home gardens sometimes can be attributed to diseased plants purchased from retail dealers and not always to the spread of the virus by leafhopper vectors after transplanting. All delphinium seedlings grown in flats offered for sale by one dealer in Oakland showed typical symptoms of aster yellows. During the spring of 1937, delphinium seedlings grown in flats were purchased from retail dealers and nurseries in Berkeley and San Pablo and were kept under observation in a glasshouse, but all plants remained healthy. Perennial delphiniums in nurseries in these two localities were usually free from aster yellows. One commercial grower in Berkeley, familiar with some symptoms of aster yellows, rogued the diseased plants.

GEOGRAPHIC RANGE OF VIRUS AND VECTORS

Discussions of host-range differences of California and New York aster yellows, overlapping host ranges, and strains of aster-yellows viruses have been published in previous papers by Kunkel (*8*), Severin (*15*), Severin and Haasis (*24*), and Smith (*26*). The results of experiments with the aster-yellows virus from eastern, middle-western, and western states have also been reported in a previous paper (*15*), and this investigation has been continued on host plants of aster yellows from other western states. The identity of the virus in other western states and the distribution of the most important vectors of the aster-yellows virus to delphinium may be worthy of discussion.

Utah.—Linford (*33, 34*) described typical symptoms of aster yellows on delphinium and also found aster yellows and a "virus-yellows" disease on celery in various localities in Utah.

Severin (*15*) demonstrated that the virus of celery yellows from Utah is probably identical with the California-aster-yellows virus.

Washington.—Heald and Burnett (*6*), mentioned previously, described typical symptoms of delphinium aster yellows occurring in the State of Washington. In a later paper, Burnett (*2*) reported that there was an excessive proliferation of the flowering stalks, which produced a bunchy witches' broom appearance. The flowering parts failed to develop normally but produced a characteristic leafy proliferation varying from slightly greenish flowers to pale-green leafy structures.

Heald[5] has never observed aster yellows in the State of Washington, and he is not convinced that delphinium aster yellows of California is identical with the disease in Washington. He states that juice inoculation from diseased to healthy delphinium plants failed to produce the proliferated inflorescence showing virescence or greening of the flowering parts. But the aster-yellows virus can only be transmitted by leafhopper vectors and by grafting and budding; it is not transmissible by juice inoculation. All attempts at this station to infect healthy Wrexham delphinium, aster, and celery plants grown from seeds by sap inoculation with the carborundum method (*12*) were failures. The inoculated delphiniums were kept under observation until the blossoming period, but none developed abnormal, green flower parts.

Glen A. Huber, Western Washington Experiment Station, Puyallup, Washington, sent 1 delphinium, 3 aster, and 2 celery plants showing typical symptoms of aster yellows. Attempts were made to recover the virus from these naturally infected host plants by means of previously noninfective leafhoppers and to transfer it to healthy seed-grown plants.

[5] Heald, F. D., in a personal interview.

Previously noninfective mountain leafhoppers recovered the virus from the naturally infected delphinium from Washington and transferred it to healthy celery plants. The virus was recovered from the experimentally infected celery plants by means of short-winged aster leafhoppers and transferred to healthy asters. Lots of 20 previously noninfective mountain leafhoppers, after feeding on the experimentally infected celery plants, were transferred to 10 healthy Pacific Giant second-year delphiniums. Three of the 10 inoculated plants developed green, abnormal flowers, numerous short chlorotic shoots grew from the crown of 5 infected delphiniums after the blossoming period, and 2 plants remained healthy. One lot each of previously noninfective short-winged and long-winged aster leafhoppers failed to recover the virus from the naturally infected delphinium and transfer it to healthy delphiniums. The delphinium plant from Washington died before further tests could be made.

Previously noninfective short-winged aster leafhoppers recovered the virus from the 3 naturally infected asters from Washington and transferred it to healthy aster and celery plants and from the experimentally infected celery plants back to asters. Repeated lots of short-winged aster leafhoppers bred on asters experimentally infected with the virus from the 3 naturally infected asters transmitted the virus to 5 Pacific Giant delphiniums; 2 plants developed abnormal flowers with green floral parts, and from the crown of 3 delphiniums grew dense clusters of short, yellow shoots after the flowering period.

The virus was also recovered from the 2 naturally infected celery plants from Washington and transferred to healthy aster and celery plants and from experimentally infected asters back to celery plants. Lots of previously noninfective geminate leafhoppers after feeding on the 2 naturally infected celery plants were transferred to 6 healthy Pacific Giant two-year-old delphiniums; 1 plant showed the green, abnormal inflorescence, and 5 developed numerous chlorotic shoots from the crowns after the blossoming period. The virus from delphinium stunt, and from aster and celery yellows from Washington is probably identical with the California aster-yellows virus.

Colorado.—C. M. Tompkins sent 9 aster-yellows plants from Brighton, Colorado. The virus was transferred from the diseased asters by short-winged and long-winged aster leafhoppers to 9 healthy aster and 3 healthy celery plants. The results indicate that the California-aster-yellows virus occurs in Colorado.

Oregon.—C. M. Tompkins sent 6 aster-yellows plants from Corvallis, Oregon. The virus was transferred by previously noninfective short-winged aster leafhoppers from the diseased asters to 5 healthy aster and

4 healthy celery plants. Thus the California-aster-yellows virus occurs in Oregon.

B. F. Dana, Corvallis, Oregon, sent a delphinium showing phyllody and virescence, but the plant died before any tests could be made.

Wyoming.—At the request of P. Brierly, United States Horticultural Station, Beltsville, Maryland, G. W. Bohn sent aster-yellows plants from Cheyenne, Wyoming. Previously noninfective short-winged and long-winged aster leafhoppers and the mountain leafhopper recovered the virus from 7 diseased asters from Cheyenne and transferred it to 11 healthy aster and 5 healthy celery plants. The virus was recovered from the 3 experimentally infected celery plants and transferred back to asters. These tests indicate that the California-aster-yellows virus also occurs in Wyoming.

Idaho.—At the request of E. C. Blodgett, Idaho Agricultural Experiment Station, C. D. Miller, Mullan, Idaho, sent 2 delphiniums showing witches' broom. Previously noninfective geminate leafhoppers transmitted the virus from the 2 diseased plants to 6 of 11 healthy two-year-old Pacific strain delphiniums but mountain leafhoppers failed to infect 15 delphiniums of the same variety.

Hungerford's (*35*) description of some of the symptoms on delphinium in Idaho are similar to those observed on delphinium naturally and experimentally infected with the aster-yellows virus in California. He states that the aborted flowering parts were very much dwarfed, but he does not mention the green leafy structures representing abnormal flower parts.

There was some variation in symptoms which developed on delphiniums experimentally infected with virus from Idaho. An apical proliferation of the spike developed with numerous 3-lobed green, leafy structures, or single-lobed leaves arranged in a rosette surrounding extremely dwarfed floral organs, or short shoots developed from the spike with linear leaves and an apical leafy cluster surrounding dwarfed flower parts.

Attempts were made to transmit the virus by means of the mountain, geminate, and short-winged and long-winged aster leafhoppers from the 2 naturally infected delphiniums from Idaho and delphiniums experimentally infected with virus from there, to healthy aster, celery, and carrot plants; but all were failures.

Severin (*15*) reported the transfer of an aster-yellows virus by means of previously noninfective short-winged aster leafhoppers from naturally infected carrots sent from Idaho to healthy carrots, but not to healthy aster and celery plants. From a second shipment of naturally infected carrots from Idaho, the virus was transferred to 3 of 61 celery

plants, but the virus was not recovered and transferred to asters from the 3 celery plants showing typical symptoms of yellows.

C. F. Henderson, of the United States Bureau of Entomology and Plant Quarantine, sent a third shipment of diseased carrots from Twin Falls, Idaho. The virus was not transferred from 10 naturally infected carrots by means of previously noninfective short-winged aster leafhoppers to 20 healthy aster and 20 healthy celery plants.

Henderson also sent 12 celery plants from Idaho, each showing a twisting of the central petioles, a symptom of California aster yellows. Previously noninfective short-winged aster leafhoppers failed to transfer the virus from the diseased celery plants to any of 12 healthy celery and 24 healthy aster plants.

The aster-yellows virus in naturally infected delphiniums, carrots, and celery from Idaho, therefore, has not been proved to be the California-aster-yellows virus.

Mountain Leafhopper. — The mountain leafhopper, *Thamnotettix montanus* Van D. has been recorded from British Columbia (*28*), Washington (*31, 32*), Oregon (*32*), California (*29, 32*), Nevada (*3*), Idaho[6], Utah (*7*), Colorado (*4, 28*) and probably occurs in most of the western states.

Geminate Leafhopper. — The geminate leafhopper, *Thamnotettix geminatus* Van D. has been recorded from Colorado (*4, 27*), Idaho (see footnote 6), Utah (*7*), California (*29, 30, 31, 32*), Washington (*11*), and Alaska (*1*). Osborn (*11*) reported that it occurred in such numbers upon clover, alfalfa, and timothy in the State of Washington, especially Pullman, as to threaten to become destructive.

It is evident that one or both of the important vectors of the aster-yellows virus to delphinium in California occur also in Washington, Oregon, Idaho, and Utah, in which states the disease of delphinium resembling aster yellows is known to occur.

Delphinium "stunt" has been reported to occur in New York (*36*), but the mountain leafhopper and the geminate leafhopper do not occur in that state. The short-winged aster leafhopper occurs in New York and it may be possible that the virus of New York aster yellows produces phyllody and virescence in delphinium.

SUMMARY

A virus disease of garden varieties of perennial delphiniums is caused by the virus of California aster yellows. The delay in the discovery of the identity of the virus was caused by the fact that the short-winged

[6] Several shipments of the mountain and geminate leafhoppers were received from Twin Falls, Idaho, collected by C. F. Henderson.

aster leafhopper, *Macrosteles divisus* (Uhl.), rarely recovers the virus from diseased delphiniums. Delphinium is an unfavorable food plant of the short-winged and long-winged aster leafhoppers; some of the leafhoppers died within 4 to 6 hours and most of them within 24 hours.

The main abnormalities resulting from aster yellows on delphiniums may be summarized as follows: (1) dwarfing of plants and a bunchy growth of short stems; (2) a general yellowing of the foliage; (3) phyllody, or the tendency of the floral organs to resemble leafy structures, and (4) virescence, or the replacement of floral pigments by chlorophyll.

The mountain leafhopper, *Thamnotettix montanus* Van D., and the geminate leafhopper, *T. geminatus* Van D., are the most important vectors of the virus to delphinium, and both species breed on this host plant under natural conditions. Adults of both species of leafhoppers were collected on naturally infected delphiniums and transferred to healthy seedlings; the mountain leafhopper transmitted the virus to 84.6 and the geminate leafhopper to 92.3 per cent of the plants. The average percentage of experimental infection of delphiniums by four vectors of the virus was as follows: two-year-old delphiniums before the spikes appeared, 86.2, and after the spikes developed, 70 per cent. The average percentages of the recovery of the virus from naturally and experimentally infected delphiniums by the different vectors were as follows: mountain leafhopper 18.3, geminate leafhopper 14.3, short-winged aster leafhopper 3.8, and long-winged aster leafhopper 11.7.

The incubation periods of the disease in seedlings of four delphinium varieties infected during June by four vectors averaged 19.5 days; in delphiniums infected during the second year in February, March, and April before spikes appeared averaged 43.5; and after spikes developed averaged 45.0 days. Delphiniums infected after the spikes appeared during the second year sometimes failed to produce green flowers with abnormal floral parts, but such plants developed a dense cluster of short, yellow shoots from the crown after the blossoming period.

The geographical range of the California-aster-yellows virus so far determined includes the following states: Oregon, Washington, Utah, Wyoming, and Colorado. The disease of delphinium resembling aster yellows is known to occur in Oregon, Washington, Utah, and Idaho, in which states either the mountain or the geminate leafhopper or both of these important vectors of the virus are known to occur.

ACKNOWLEDGMENTS

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APPENDIX TO CITATIONS

Brief notes of the occurrence of delphinium virus diseases in the United States have appeared in the *Plant Disease Reporter*.[7] Frequently the collaborators of these reports were not mentioned, and it was found more convenient to list them in the chronological order rather than under the name of collaborators and editors.

33. The Plant Disease Reporter Sup. 59:111. 1927.
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35. The Plant Disease Reporter 17:5. 1933.
36. The Plant Disease Reporter Sup. 90:135. 1935.
37. The Plant Disease Reporter Sup. 96:268–69. 1936.
38. The Plant Disease Reporter Sup. 110:263. 1938.

[7] A mimeographed pamphlet issued by the United States Department of Agriculture Bureau of Plant Industry.

PLATES

Plate 1.—*A*, One shoot from a dense cluster about 6 inches high showing lateral or axillary shoots with bunched, abnormal leaves, and elongated, chlorotic petioles, also linear or filamentous leaves and threadlike petioles; *B*, Wrexham delphinium seedling experimentally infected with aster yellows showing greatly enlongated, vertical, frequently curved, white petioles with dwarfed, chlorotic leaves; *C*, lower center, normal leaf with 3 lobes or divisions, others, abnormal leaves with lobes partly developed, or 1 or 2 lobes absent; *D*, naturally infected seedling showing symptoms similar to *B* except that the petioles were shorter and most of the leaves were cupped inward. The greatly elongated petioles, such as those in *B*, developed only when kept in cages in the greenhouse.

Plate 2.—*A*, Sectorial infection showing normal, colored flowers and abnormal flowers with green, leafy sepals and petals on opposite sides of the spike; *B*, cluster of green, leafy structures replacing normal sepals, petals, carpels, and stamens; *C*, one of many slender shoots which may remain dwarfed until the blossoming period, or may develop a slender spike—the petioles are elongated and yellow, often with chlorotic leaves cupped inward; *D*, chlorotic leaves from a delphinium plant experimentally infected with aster yellows during the second year before spike developed, showing cupped blades and curved petioles.

Plate 3.—Apical end of spikes with abnormal flowers from delphinium plants naturally infected with aster yellows: *A*, flower buds which were green; *B*, enlarged green, leafy sepals and dwarfed petals; *C*, replacement of carpels by petiolate leaflike structures or stems; *D*, left, lower flowers which were green instead of blue, upper flowers with elongated stamens and leafy, petiolate, partly opened carpels, which in some cases have lobes resembling ovules; right, two flowers with sepals, petals, carpels, and stamens replaced by green, leafy structures.

Plate 4.—Abnormal floral parts from delphiniums naturally and experimentally infected with California aster yellows: *A*, cupped sepals, dwarfed petals, and elongated stamens; *B*, enlarged sepals and abnormal floral parts; *C*, 3-lobed leaves and central dwarfed flower parts; *D*, *F*, enlarged, cupped sepals, dwarfed petals, and replacement of carpels by structures resembling leaves; *E*, structures resembling small flower buds; *G*, one stalked bud arising in the center of another bud.

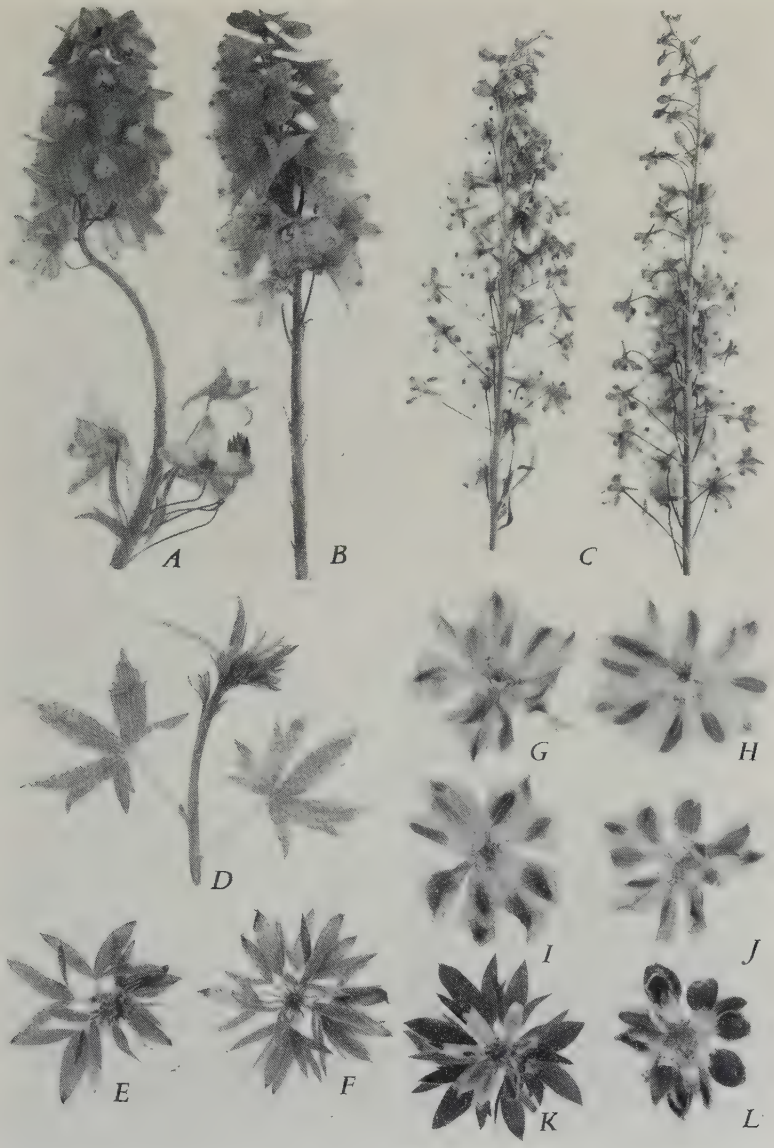

Plate 5.—Abnormal flowers of delphiniums naturally infected with California aster yellows: *A*, cluster of apparently normal flowers on the apical and basal regions of spike with the intermediate part bearing filamentous structures; *B*, cluster of flowers restricted to apical portion of spike; *C*, dwarfed flowers with elongated pedicels; *D*, spike showing apical cluster of abnormal flowers surrounded by dwarfed single-lobed leaves and with lower, normal divisions of leaves; *E*, *F*, rosettes with 3-lobed leaves surrounding undeveloped floral structures; *G–J*, flowers showing median green areas on petals; *K*, rosette composed of 3-lobed leaves, dwarfed sepals, and petals; *L*, rosette with cupped leaves and sepals, and dwarfed petals.

Plate 6.—Proliferation of apical end of spike from delphiniums naturally infected with California aster yellows: *A*, dense cluster of leaves enclosing green flowers; *B*, cluster of leafy structures replacing normal sepals, petals, carpels, and stamens, with lower, normal-shaped leaves; *C*, linear structures representing abnormal floral parts with lower single-lobed leaves; *D*, bunchy, tangled mass of flower parts; *E*, cluster of wilted leaves from fallen stalk still adhering to roots.

Plate 7.—Apical ends of spikes from delphiniums naturally infected with California aster yellows: *A*, central cluster of abnormal flowers with filamentous flower parts and dwarfed apical leaves surrounding abnormal green flowers; *B*, abnormal, green flowers with filamentous parts arranged and spaced on the spikes like those of healthy plants; *C*, dwarfed, green flowers with long pedicels on apical end of spike with lateral branches surrounded by linear leaves; *D*, numerous slender branches with dwarfed, abnormal, green flowers suggesting a witchs'-broom appearance.

Plate 8.—Dreer's De Luxe delphinium naturally infected with California aster yellows with necrotic symptoms on the stems, petioles, and blades, probably caused by secondary bacterial infections and not directly by the aster-yellows virus: *A*, black, necrotic streaks on stem and on some of the petioles; *B*, black, necrotic tissue on blade; *C*, black, necrotic areas at the attachment of some of the petioles and on some of the blades; *D*, stem showing small, brown, necrotic lesions; *E*, long necrotic streak; *F*, cracks; *G*, black lesions which girdle the stem; *H*, spike showing apical cluster of abnormal flowers, black stem, and dead leaves.

CELERY CALICO ON PERENNIAL DELPHINIUMS AND CERTAIN OTHER HOST PLANTS

HENRY H. P. SEVERIN

CELERY CALICO ON PERENNIAL DELPHINIUMS AND CERTAIN OTHER HOST PLANTS[1]

HENRY H. P. SEVERIN[2]

INTRODUCTION

One of the viroses found in the course of the investigations of aster yellows on perennial delphiniums (*4*)[3] proved to be transmissible by juice inoculation and showed symptoms resembling those of calico on celery. Calico was reported by Severin and Freitag in 1935 (*5*). In a later paper (*6*) they described and figured the symptoms of the disease. But the disease has not hitherto been reported on delphiniums or any other species of the family Ranunculaceae.

An investigation was accordingly undertaken to determine whether one of the naturally occurring viroses of perennial delphiniums was caused by the celery-calico virus. Studies were made of the variable symptoms of the disease on this host plant, the incubation period of the disease, the recovery of the virus, and the vectors. A number of hybrids and horticultural varieties of perennial delphiniums were tested for susceptibility to celery calico. A few other host plants of the virus are reported in this paper.

Delphiniums in the field in California were found to be frequently infected with more than one virosis—as, for example, with aster yellows and the disease reported in this paper. This situation greatly complicates the problem, especially since the symptoms of these viroses are extremely variable, even when they occur singly. Little progress can be made unless multiple viruses can be separated, and attempts were therefore made to work out methods for doing this in delphinium and also in tomato. Much confusion in the literature dealing with delphinium viroses is caused by the failure of some plant pathologists to recognize and separate multiple viruses in naturally infected plants.

Heald and Burnett (*2*), working with delphiniums infected with what they called "stunt" (aster yellows) (*4*), reported that some of the symptoms were reproduced by juice inoculation to healthy transplants and seedlings grown in the greenhouse, but that the inoculated plants did not develop the proliferated inflorescence and virescence shown by the naturally infected plants. They state, "It seems probable that all of the

[1] Received for publication May 10, 1941.

[2] Associate Entomologist in the Experiment Station.

[3] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

observations are concerned with slightly different phases of a single disease although it is possible that more than a single virus disease is represented."

Burnett (*1*) states that healthy delphiniums inoculated with "macerated leaf tissue" from naturally infected delphiniums produced such symptoms as ringspot, necrosis, and chlorosis. Plants from which the virus was secured exhibited such symptoms as necrosis, chlorosis or yellowing, a witches'-broom effect, and a reversion of the floral parts to green, leafy structures. He assumed that a single virus produced extremely variable symptoms on delphinium and some other host plants, under different environmental conditions. In the present investigation, some of the host plants reported by Burnett for delphinium stunt were inoculated with celery-calico virus to test susceptibility and compare the symptoms with those described by Burnett.

MATERIAL AND METHODS

The sources of inoculum were delphinium, celery, cantaloupe, cucumber, Summer Crookneck squash, and tomato, all naturally infected with calico. The virus extract from these host plants was usually inoculated in the cotyledons of healthy cucumbers, and the virus was retained by repeated mechanical inoculation to cucumbers. Cucumbers were used because the symptoms developed rapidly on the cotyledons, often in 2 days. The carborundum method of inoculation described by Rawlins and Tompkins (*3*) was used.

The multiple viruses in naturally infected delphinium and tomato were separated by means of filter plants. The aster-yellows virus was separated from a virus complex in delphinium by means of previously noninfective leafhoppers.

SYMPTOMATOLOGY

The symptoms of celery calico on delphinium are very conspicuous in the field and can be noticed across three or four rows of plants. The basal or lower and intermediate leaves show pale-orange, amber, or lemon-yellow, irregular areas, *but the younger leaves never show symptoms of the disease.*

A closer examination of the leaves showing symptoms from perennial delphiniums naturally and experimentally infected with calico reveals a considerable amount of variation in the patterns. A few of the oldest leaves of infected seedlings may show irregular, chlorotic areas on some or all of the lobes or divisions (plate 1, *A, B*), or an entire leaf may assume a pale-orange or lemon color. Variations in the patterns may occur on the older leaves of the same plant, such as irregular discolora-

tions on one leaf (plate 1, *C*) and small green areas scattered in the chlorotic region of another leaf (plate 1, *D*). Such green islands in the pale-orange, amber, or lemon-yellow areas (plate 1, *F*) are reliable symptoms of calico on celery and other host plants. Sometimes a lower leaf of an infected delphinium seedling may show chlorotic or green streaks (plate 1, *E;* plate 2, *D*).

Both naturally and experimentally infected delphiniums frequently show line or ring patterns. The lines are often broken, consisting of a series of chlorotic dots or dashes (plate 2, *A, B*), or alternating green and yellow lines (plate 2, *C*). Ring patterns resembling ringspots may be composed of chlorotic dots enclosing green areas (plate 2, *E*); sometimes the chlorotic dots are arranged in groups. The rings may consist of concentric, alternating, yellow and green lines surrounding green centers (plate 2, *F*).

The Chinese varieties of delphiniums (*Delphinium grandiflorum*) developed chlorotic, lateral shoots and the linear segments of the blades were yellow and failed to develop the variable symptoms previously described.

The virus of calico causes breaking in the color of pansies and violas (plate 6, *K*), and this symptom is one method of identifying the virus; but the blossoms of naturally and experimentally infected delphinium were normal in appearance.

EXPERIMENTAL INFECTION

Since a considerable amount of variation occurs in the patterns on the leaves of delphinium plants naturally and experimentally infected with calico, inoculations of delphiniums were made with the virus extract from a number of naturally infected host plants obtained in various localities in California. Delphiniums showing symptoms of calico were collected in the San Francisco Bay district, Mt. Eden, Capitola, Hillsborough, Salinas, and Fresno. The expressed juice from delphiniums showing different calico patterns on the leaves was inoculated in healthy delphiniums, cucumber, and Turkish tobacco, and subinoculations were made from the last two plants back to healthy delphinium seedlings. Inoculations of healthy delphinium seedlings were also made with the virus extract from other naturally infected host plants of calico as follows: cantaloupe from Sacramento Pocket, celery from Milpitas, cucumber (plants and fruit) from San Pablo, Summer Crookneck squash (fruit) from Santa Cruz, and tomato from Berkeley. The results are shown in table 1. The inoculum from delphiniums and the various host plants of calico produced variable symptoms on the leaves of delphiniums regardless of the source of virus.

TABLE 1

INCUBATION PERIOD OF DISEASE IN DELPHINIUMS INOCULATED WITH VIRUS EXTRACT FROM HOST PLANTS NATURALLY INFECTED WITH CALICO

Source of inoculum (host plant and district), delphinium variety inoculated, and date of inoculation (1936–1940)	Plants inoculated	Plants infected	Incubation period of disease in plants	
			Range	Mean
	number	*number*	*days*	*days*
From delphinium at Fresno:				
Into Wrexham delphinium:				
September 21	5	3	65–77	71 0
February 16	5	1	39	....
From delphinium at Berkeley:				
Into Wrexham delphinium:				
September 23	5	1	152	....
From delphinium at Montara:				
Into Wrexham delphinium:				
October 4	5	2	67–87	71.7
December 11	5	2	18–50	34.0
February 2	5	2	25–25	25.0
From delphinium at Colma:				
Into Wrexham delphinium:				
November 6	5	1	75	....
December 2	5	1	106	....
From cantaloupe at Sacramento Pocket:				
Into Wrexham delphinium, September 23	5	1	178	....
Into tall hybrid Bellamosum delphinium:				
March 5	5	1	40	....
From celery at Milpitas:				
Into Blackmore and Langdon delphinium:				
October 1	5	4	40–46	44.5
October 5	5	1	42	....
October 6	5	2	41–49	45.0
October 7	10	7	28–35	33.0
Into White Elatum (Summer Cloud) delphinium:				
October 7	5	1	28	....
From cucumber at San Pablo:				
Into Wrexham delphinium:				
March 24	5	4	39–55	47.0
From Summer Crookneck squash at Santa Cruz:				
Into Wrexham delphinium:				
September 23	5	3	131–159	145.3
From tomato at Berkeley:				
Into Wrexham delphinium:				
September 26	5	1	143	....
December 15	5	2	18–79	57.7

TABLE 2

LIST OF VARIETIES AND HYBRID DELPHINIUM SEEDLINGS EXPERIMENTALLY INFECTED WITH CELERY CALICO, INCUBATION PERIOD OF DISEASE, AND RECOVERY OF VIRUS

Delphinium variety or hybrid and date inoculated (1936–1940)	Delphiniums inoculated	Delphiniums infected	Incubation period of disease in delphiniums		Recovery of virus from infected delphiniums	
			Range	Mean	Cucumbers inoculated	Cucumbers infected
Blackmore and Langdon hybrids:	*number*	*number*	*days*	*days*	*number*	*number*
June 28	2	2	19–19	19.0	10	6
August 31	5	3	23–38	30.0	15	15
Belladonna tall hybrids:						
May 6	3	3	30–39	34.0	15	9
June 12	3	3	17–24	21.0	15	15
July 30	4	3	37–66	56.3	15	15
Chinensis grandiflorum var. *album:*						
June 12	5	5	26–43	34.0	25	25
Chinese Azure Blue:						
June 24	3	2	20–33	26.5	10	10
August 31	2	1	69	....	5	0
Chinese Dark Blue:						
June 12	5	5	43–43	43.0	25	25
Clivenden Beauty:						
June 12	5	5	15–31	22.5	25	25
Delphinium Parryi var. *maritimum:*						
November 25	1	1	—*	—*	5	5
Delphinium Zalil:						
March 12	1	1	—*	—*	5	5
Dreer's De Luxe Art shades:						
June 12	5	5	15–28	20.8	25	25
Dreer's De Luxe Dark-Blue shades:						
June 12	5	5	16–43	25.0	25	25
September 17	1	1	52	....	5	5
October 15	3	3	—*	—*	15	15
Dreer's De Luxe Light-Blue shades:						
June 28	3	2	23–26	24.0	10	10
September 17	1	1	82	....	5	2
Dreer's De Luxe Mid-Blue shades:						
August 31	5	1	35	....	5	0
June 12	5	5	16–17	16.2	25	25
Dwarf Chinese Butterfly:						
June 12	5	2	15–28	21.5	10	10
English hybrids Deep-Blue shades:						
June 12	5	5	15–17	16.0	25	25
English hybrids Mid-Blue shades:						
June 12	5	3	15–17	15.7	15	15
October 15	5	1	41	....	5	5
English hybrids Pastel shades:						
June 28	3	3	13–26	21.0	15	15
Burpee's Floradale Giants Deep Blue:						
June 29	1	1	—*	—*	5	5
August 31	5	3	23–26	25.0	15	0
Burpee's Floradale Giants Light Blue:						
June 28	2	2	14–14	14.0	10	10
August 31	1	1	40	....	5	5
September 17	2	2	23–45	34.0	10	10
Burpee's Floradale Giants Mid Blue:						
June 24	3	3	15–31	21.0	15	0
August 31	5	3	23–40	32.3	15	0
September 17	1	1	52	....	5	5

* No symptoms, but virus recovered from infected delphiniums.

(Table concluded on next page)

TABLE 2—(*Concluded*)

Delphinium variety or hybrid and date inoculated (1936–1940)	Delphiniums inoculated	Delphiniums infected	Incubation period of disease in delphiniums		Recovery of virus from infected delphiniums	
			Range	Mean	Cucumbers inoculated	Cucumbers infected
	number	*number*	*days*	*days*	*number*	*number*
Burpee's Floradale Art or Pastel shades:						
October 2	4	4	—*	—*	20	20
October 15	1	1	24	....	5	4
Giant Single and Double hybrids:						
June 24	3	3	18–30	22.0	15	12
A. & M. Gold Medal hybrids:						
June 12	5	5	15–31	22.8	25	25
Gold Medal hybrids:						
June 24	5	4	12–15	13.0	20	20
September 17	1	1	52	....	5	5
October 15	4	1	—*	—*	5	2
Hardy larkspur (*Delphinium formosum*):						
June 12	5	5	15–21	17.2	25	25
August 31	5	3	23–35	27.0	15	15
Hybridum mixed:						
June 12	5	5	15–17	15.6	25	25
October 2	4	1	23	....	5	5
Iceberg:						
June 12	5	4	17–17	17.0	20	20
July 31	5	1	59	....	5	5
Improved Belladonna Clivenden Beauty:						
October 2	5	1	37	....	5	5
Improved English hybrids:						
June 24	5	4	12–30	19.6	20	20
September 17	1	1	45	....	5	5
October 15	2	2	—*	—*	10	4
November 25	1	1	—*	—*	5	4
Lady Guinevere:						
September 17	3	3	—*	—*	15	12
October 2	1	1	37	....	5	0
Lemon Gem:						
June 24	5	4	—*	—*	20	20
Burpee's Mammoth hybrids:						
June 24	5	5	11–14	12.0	25	25
New Hollyhock strain:						
September 17	3	3	28–45	33.7	15	15
Pacific Giant mixed:						
June 12	5	4	17–44	28.0	20	20
Pacific Giant White:						
June 12	5	3	23–31	26.3	15	15
June 12	2	2	—*	—*	10	4
September 17	2	2	63–63	63.0	10	10
September 17	3	3	—*	—*	15	15
A. & M. Sunbeam hybrids:						
June 24	5	5	16–16	16.0	25	25
Wrexham Hollyhock strain:						
September 17	2	2	28–63	45.5	10	10

* No symptoms, but virus recovered from infected delphiniums.

Varieties and Hybrids Inoculated.—The delphinium varieties and hybrids infected with the inoculum from various host plants naturally and experimentally with calico, are listed in tables 1 and 2. All delphiniums listed in these tables were seedlings except the Wrexham, or Hollyhock, variety (table 1), which was used after the second year's growth.

Incubation Period of the Disease.—The period from the date of inoculation until the orange or yellow discoloration, line, or ring patterns appeared on the basal leaves of delphinium seedlings was considered as the incubation period of the disease.

As indicated in tables 1 and 2, the incubation period of the disease varied from 11 to 178 days.

During the past year, Pacific-strain second-year delphiniums infected with calico were marked in the field in Berkeley, and it was noted that in the new shoots appearing from the ground after the old stock was cut off, symptoms on the leaves were slower to develop in the summer than in the spring, and still slower in the fall. During the past mild winter (1941–42), the leaves on the new shoots failed to show symptoms even after the spikes developed.

Recovery of the Virus.—An attempt was made to recover the virus from some of the experimentally infected delphiniums during the second year. Fifteen delphinium seedlings were selected which showed prominent and characteristic symptoms of the disease and from which the virus had been recovered during the first year. All of the foliage was removed during November, and the roots were given a rest period during the winter. After a period of 9 to 12 months had elapsed since the seedlings had been inoculated, the juice was expressed from the new shoots and inoculated in Turkish tobacco and cucumber plants. The virus was not recovered from 1 delphinium plant which showed typical symptoms of the disease during the first and second years. But it was recovered from the other 14 experimentally infected plants, 4 of which failed to show symptoms of the disease during the second year. Evidently some infected delphinium plants are symptomless carriers of the disease during the second year.

Delphinium plants which appeared to be infected with a cucumber mosaic were found under natural conditions; others revealed a mottling of the leaves resembling a mosaic disease, and many showed dried, brown flowers with or without malformed leaves. Repeated cross inoculations were made from these abnormal plants (transplanted in a glasshouse) to healthy delphinium seedlings, but all attempts to reproduce the symptoms were failures. Frequently these abnormal plants also showed symptoms of aster yellows or sometimes calico. The virus of

calico was sometimes recovered from these abnormal plants, which failed to show symptoms of calico on the leaves. Evidently some of these abnormal plants were symptomless carriers of calico and served as reservoirs of the virus. It is not to be inferred, however, that the calico virus produced the abnormalities.

HOST RANGE

In this paper the symptoms of calico on a few cultivated plants are described, and compared with the symptoms described by Burnett (*1*). According to Burnett (*1*) the host of range of delphinium "stunt" (aster yellows) includes 12 species of plants: Connecticut Havana tobacco, tomato, cucumber, petunia, zinnia, and 7 species of weeds. The source of virus was from naturally infected delphiniums transplanted in boxes in the greenhouse.

Tobacco.—The earliest symptom of calico on Turkish tobacco, *Nicotiana Tabacum,* infected with the virus extract from diseased celery or delphinium plants is broken, concentric, necrotic, circles (plate 3, *A*) which appear in 4 days or longer on the inoculated leaves. The circles are composed of necrotic dots (plate 3, *B*) when examined under a binocular microscope. The next symptom on the inoculated leaves is the oak-leaf pattern (plate 3, *C*) consisting of necrotic dots and lines. A striking oak-leaf pattern also developed on the leaves of *N. Langsdorfii* (plate 3, *D*). In the advanced stage of the disease, the entire inoculated leaf may show variable ring and oak-leaf patterns (plate 3, *E, F*). The necrotic ring and oak-leaf patterns are confined usually to the inoculated leaves and sometimes to a few subsequently developing leaves. As infected Turkish tobacco plants grow older, the inoculated leaves and those above the inoculated ones exhibit intervenal, circular, chlorotic areas (plate 4, *A*), which coalesce and form irregular, blotchy mottle (plate 4, *B*) that extend to near the midrib (plate 4, *C, D*). The leaves below the flowers may show faint, chlorotic areas (plate 4, *E, F, G*) or no symptoms.

Burnett (*1*) described and figured a white, necrotic, etching, with ring-and-line pattern of variable forms confined to the first few leaves on Connecticut Havana tobacco, *Nicotiana Tabacum,* as a result of inoculation from delphiniums affected with "stunt." He also described and figured a blotchy, irregular, usually intervenal mottle which may coalesce to form extensive chlorotic areas involving the major part of the older leaves. These symptoms correspond to those produced by the virus of calico on Turkish tobacco. This indicates that Burnett probably was transmitting the calico virus from the delphiniums affected by a combination of the aster-yellows and calico viruses, since some of the symptoms on delphiniums were those of aster yellows (see p. 412).

Valleau (*7*) described and figured a ring-and-line pattern resembling calico on the leaves of Turkish tobacco rubbed with the extract from a virus disease of delphinium in Kentucky.

White Spine Cucumber.—The first symptom that often develops on the cotyledons of infected seedlings of White Spine cucumber, *Cucumis sativus,* 2 days or longer after inoculation, is large, pale-green circular areas which later are surrounded by chlorotic rings (plate 5, *A*). The next symptom which appears on the younger leaves is numerous, small, circular, chlorotic spots (plate 5, *B*), which coalesce and form irregular, yellow areas (plate 5, *C*) that spread over the leaves. The petiole is bent and the youngest leaves are sometimes cupped inward. In a late stage of the disease, the older leaves become chlorotic except for bands of green tissue extending along some of the veins (plate 5, *D*). Cucumber fruits show irregular, chlorotic mottling (plate 5, *E, F*).

The symptoms on cucumber described by Burnett (*1*) as resulting from inoculation from delphinium are not those produced by the virus of calico. He reports that the fruit which matured failed to show evident symptoms.

Marglobe Tomato.—The symptoms of calico on Marglobe tomato plants, *Lycopersicon esculentum,* begin on the lower or older leaves of tomato plants, and appear as an orange discoloration on a portion of the leaflets (plate 6, *A*) and spread until the entire leaf is affected. A progressive orange discoloration of the lower leaves on the main stem and lateral branches continues, but the younger leaves remain green. This symptom of calico may be readily overlooked, since a natural yellowing of the lower leaves occurs on healthy check or control plants, especially on old plants. Sometimes green islands occur in the orange discoloration.

The symptoms on tomato described by Burnett (*1*) do not correspond to those produced by the virus of calico. He states, however, that fern leaf is not caused by the delphinium virus alone.

Rosy Morn Petunia.—The first symptom of the disease which appeared on Rosy Morn petunia, *Petunia hybrida,* was a clearing of the veins (plate 6, *C*) 6 days or longer after inoculation with the virus extract from delphinium infected with celery calico. In the later stage of the disease, the basal or lower leaves become orange in color; sometimes the tissue along the midrib remains green. The older ascending leaves develop variable orange and green patterns (plate 6, *F, G, H*), sometimes deep-green blotches in the chlorotic tissue, or an oak-leaf pattern along the midrib and lateral veins (plate 6, *D, E*).

Symptoms were not evident on *Petunia hybrida,* according to Burnett, (*1*) but the virus was recovered when subinoculations were made to tobacco plants.

MULTIPLE VIRUSES

It seems likely that Burnett (*1*) was dealing with multiple viruses in his host-range studies. He unquestionably failed to reproduce the symptoms of aster yellows on delphinium by juice inoculation since this virus is inactivated in expressed juice (*4*). Possibly the inoculum from naturally infected delphiniums may have contained the calico virus. His inoculations into Connecticut Havana tobacco indicate this to be the case but his inoculations into other host plants do not substantiate this interpretation.

A delphinium plant sometimes contains both calico and aster-yellows viruses in the field, and the inoculum from such a plant produces infection of calico but not aster yellows. The aster-yellows virus was recovered from the virus complex in delphinium plants by previously noninfective mountain leafhoppers, *Thamnotettix montanus* Van D., geminate leafhoppers, *T. geminatus* Van D., and the long-winged aster leafhoppers, *Macrosteles divisus* (Uhl.).

Tomato plants are frequently infected with a mixture of viruses under natural conditions; for example, ordinary tobacco mosaic identical with tomato mosaic (tobacco virus 1) and calico. The virus of ordinary tobacco mosaic sometimes produces symptoms described as fern leaf, filiform leaf, or shoestring leaf (plate 6, *I*), when young, slow-growing tomato plants are inoculated and kept under low temperatures and light intensity, but the calico separated from the virus complex rarely induces such symptoms. When the multiple viruses in the tomato extract are inoculated in cucumber plants, tobacco mosaic is filtered out and the calico virus is retained.

GEOGRAPHIC RANGE OF THE VIRUS

California.—Perennial delphiniums naturally infected with the celery-calico virus were collected in the San Francisco Bay districts, in the Santa Clara, Salinas, and San Joaquin valleys, and in Capitola, Montara, and Hillsborough. Celery calico has been found in all of the large celery districts of California.

Washington.—G. A. Huber, Western Washington Experiment Station, Puyallup, Washington, kindly sent 2 celery plants showing typical symptoms of calico. The virus extract from these 2 plants was mechanically inoculated into five known hosts of celery calico, 5 plants being used in each test. The numbers of these that became infected were: Wrexham delphinium 5, celery 2, White Spine cucumber 5, Turkish tobacco 3, and *Nicotiana glutinosa* 3. It is evident that the virus of celery calico from Washington is identical with that from California.

Idaho.—One of two delphinium witches' broom (aster yellows) (*4*) sent from Mullan, Idaho, by C. D. Miller, showed typical symptoms of calico while the other plant was a symptomless carrier of the virus. The virus extract from these two plants inoculated into 5 known hosts of celery calico produced the following infections: 5 Blackmore and Langdon delphiniums, 18 celery, 29 White Spine cucumbers, 5 Turkish tobacco, and 5 *Nicotiana glutinosa.* The results indicate that the virus separated from this virus complex is probably identical with the virus of celery calico from California and the range of the virus may thus be tentatively extended to Idaho.

APHID VECTORS

The natural occurrence of colonies of aphids on delphinium plants has never been observed in California by the author. Occasional winged aphids were found on delphiniums under natural conditions, but most of these were dead.

It was difficult to infect delphinium seedlings with calico by means of any of the species of aphids tested, even though each plant was inoculated with the virus by two lots of 40 aphids each. The following species of aphids reared on celery infected with calico, did, however, transmit the virus to healthy delphinium seedlings to which they were transferred:

Celery leaf aphid, *Aphis apigraveolens* Essig
Celery aphid, *Aphis apii* Theobald
Rusty-banded aphid, *Aphis ferruginea-striata* Essig
Cotton, or melon, aphid, *Aphis gossypii* Glover
Erigeron root aphid, *Aphis middletonii* Thomas
Lily aphid, *Myzus circumflexus* (Buck.)
Foxglove aphid, *Myzus convolvuli* (Kalt.)
Green peach aphid, *Myzus persicae* (Sulz.)
Honeysuckle aphid, *Rhopalosiphum melliferum* (Hottes)

The virus was recovered by previously noninfective cotton, or melon, aphids, *Aphis gossypii,* from a delphinium plant infected by this species of aphid and transferred to a healthy celery plant. No difficulty was experienced in recovering the virus from delphiniums infected with the nine listed species of aphids by mechanical inoculation of the virus extract into healthy delphinium, celery, and cucumber plants.

Winged green peach aphids occasionally were taken on delphiniums grown in the field, and an attempt was made to recover the virus by previously noninfective aphids from naturally infected delphiniums and transfer it to healthy plants. The virus was transmitted to 2 of the 17 plants that were inoculated. A high mortality of the aphids occurred on delphiniums.

SUMMARY

A virus disease of perennial delphinium has been proved to be celery calico.

The symptoms of celery calico on delphiniums are confined to the basal and intermediate leaves and are variable, including pale-yellow, amber, or lemon-yellow areas, or line or ring patterns. The virus does not cause abnormal flowers or breaking in the color of the flowers.

The incubation period of the disease ranged from 11 to 178 days.

Delphiniums either naturally or experimentally infected during the first year may be symptomless carriers of the virus during the second year.

Calico is often associated with aster yellows in delphiniums under natural conditions, and the inoculum from such plants produces infection of calico but not aster yellows. The aster-yellows virus was recovered from the virus complex by three species of leafhoppers.

Tomato plants are sometimes infected with a mixture of viruses. Cucumber plants, when inoculated with the virus extract, filter out ordinary tobacco mosaic virus and retain the calico virus.

Nine species of aphids were demonstrated to be vectors of the virus.

ACKNOWLEDGMENTS

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PLATES

Plate 1.—Delphinium leaves showing symptoms of celery calico from plants grown from seeds and infected with the virus by mechanical inoculation: *A*, leaf showing irregular, yellow discolorations on three lobes or divisions from a seedling infected with the virus extract from Summer Crookneck squash (fruit) naturally infected with calico; *B*, leaf from a delphinium seedling inoculated with the juice extracted from the same squash, showing yellow areas extending into all lobes; *C*, *D*, two leaves showing variation in symptoms from the same delphinium seedling inoculated with the juice from celery calico, *C* showing leaf discoloration and *D* green areas in the chlorotic regions; *E*, leaf showing yellow speckling and streaks from a seedling inoculated with the virus extract from a tomato plant naturally infected with calico; *F*, leaf showing green islands in the lemon-yellow areas from a seedling inoculated with the expressed juice from a delphinium plant naturally infected with the disease.

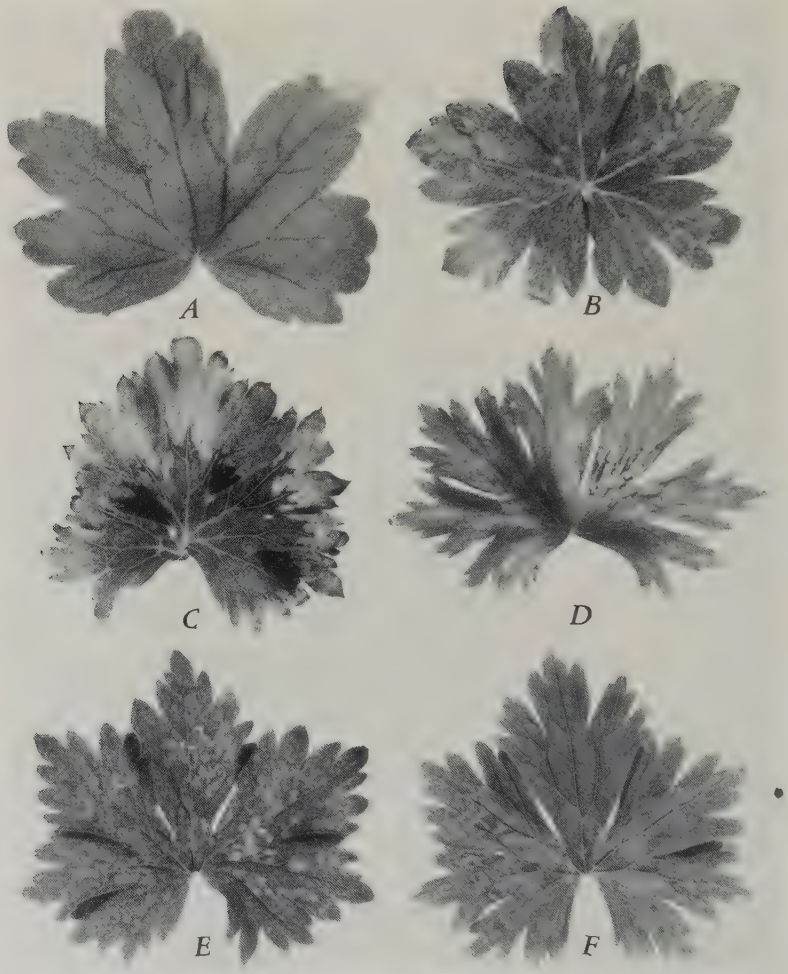

Plate 2.—Delphinium leaves showing symptoms of celery calico from plants grown from seeds and experimentally infected by mechanical inoculation: *A*, *B*, leaves from delphinium seedlings inoculated with the virus extract from celery calico, showing broken-line patterns composed of a series of dots or dashes; *C*, margin of leaf showing alternating green and yellow lines from a seedling inoculated with the juice extracted from a delphinium plant naturally infected with calico; *D*, leaf showing green streaks in the chlorotic areas from a seedling inoculated with the extract from Summer Crookneck squash (fruit) naturally infected with calico; *E*, *F*, leaves from delphinium seedlings inoculated with the expressed juice from celery calico showing ring patterns composed of chlorotic dots encircling green areas or concentric, alternating yellow and green lines surrounding green centers.

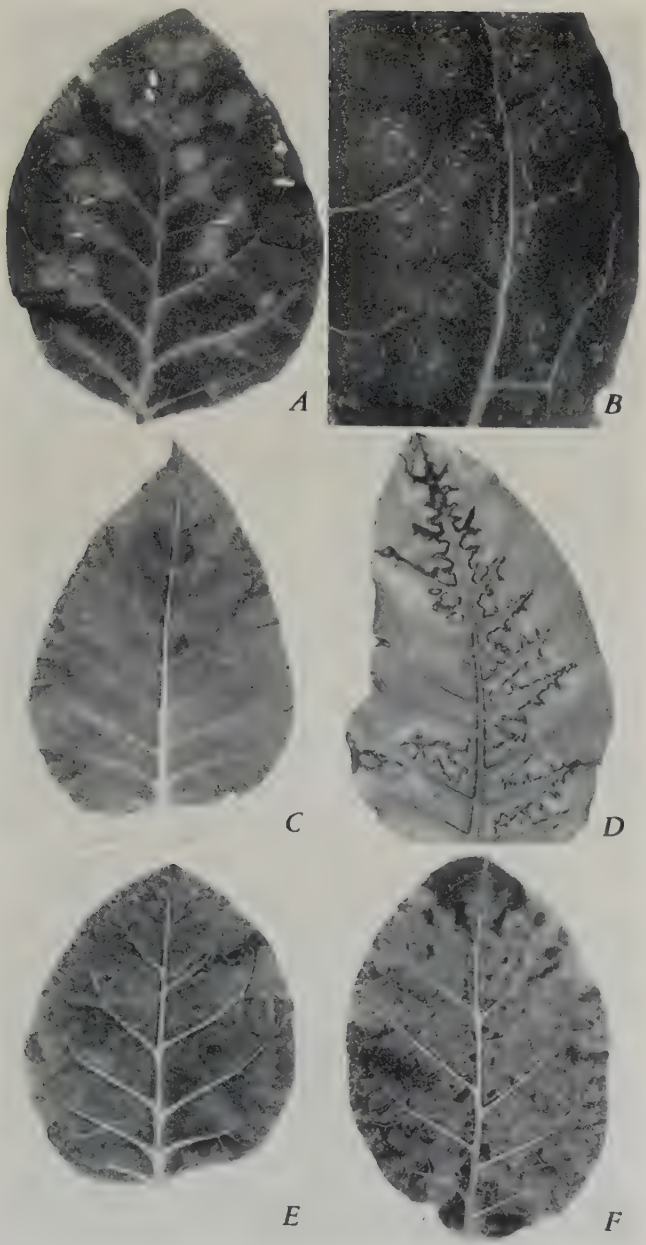

Plate 3.—Leaves from Turkish tobacco (*Nicotiana Tabacum*) and *N. Langsdorfii* infected with the celery-calico-virus extract from celery or from delphinium by mechanical inoculation: *A*, concentric, necrotic, broken circles—white areas are abrasions caused by inoculation with carborundum; *B*, portion of Turkish tobacco leaf enlarged showing necrotic dots arranged to form concentric, broken, ring patterns; *C*, etch or oak-leaf pattern consisting of necrotic dots and lines; *D*, leaf from *N. Langsdorfii* showing oak-leaf pattern; *E*, leaf from Turkish tobacco in an advanced stage of the disease showing chlorotic rings encircling green areas and line patterns; *F*, intervenal, necrotic, etch pattern.

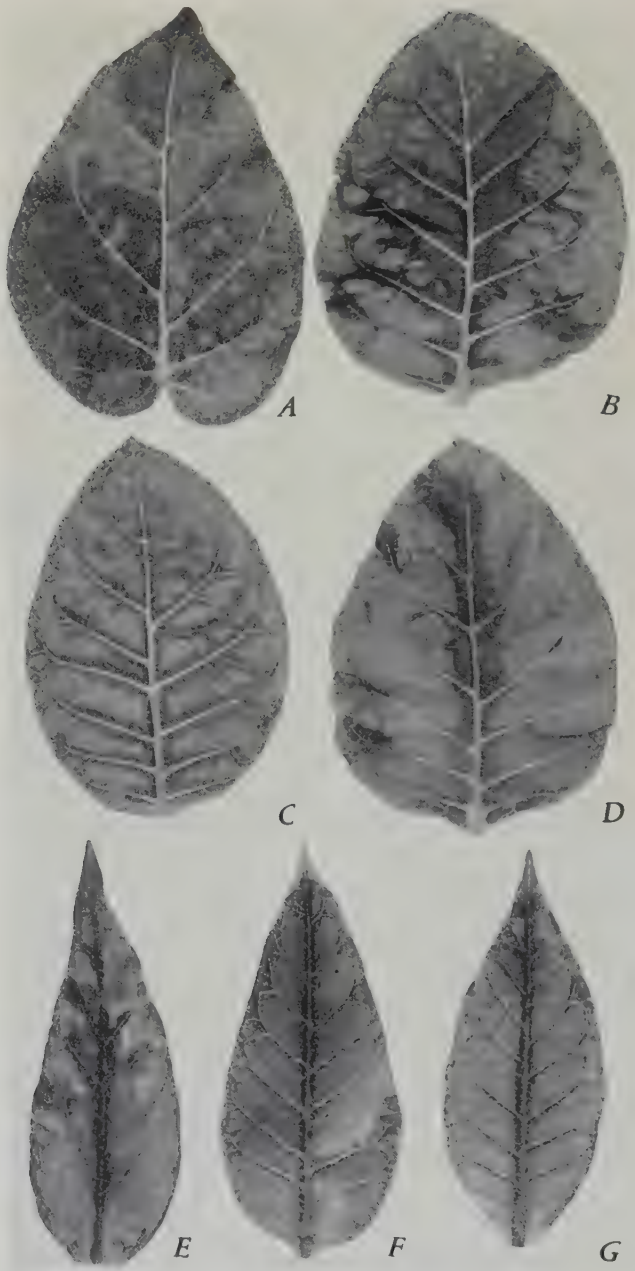

Plate 4.—Leaves from Turkish tobacco (*Nicotiana Tabacum*) infected with the virus of celery calico from delphinium by mechanical inoculation: *A*, intervenal, chlorotic, circular areas which coalesce and form irregular, blotchy mottle, as at *B*, that often extend to the midrib, as at *C*, *D*; *E*, *F*, leaves below flowers showing faint chlorotic areas; *G*, chlorosis extending over almost the entire leaf.

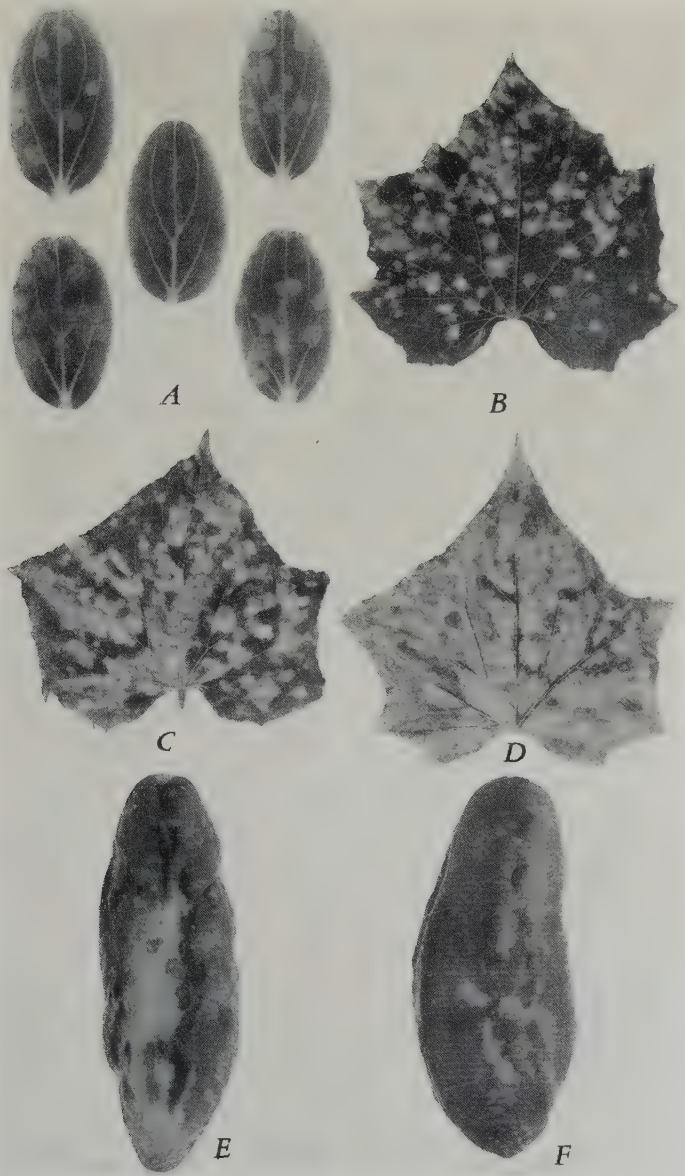

Plate 5.—White Spine cucumber (*Cucumis sativus*) infected with the virus of celery calico from delphinium by mechanical inoculation: *A*, center, cotyledon from check or control plant and grouped around it four cotyledons showing chlorotic rings enclosing large green areas; *B*, leaf showing numerous, small, circular, chlorotic spots, which coalesce to form irregular, yellow areas, as at *C*, that spread over the leaf; *D*, leaf showing advanced stages of chlorosis with green bands of tissue extending along some of the veins; *E*, *F*, cucumber fruits showing irregular, chlorotic mottling.

Plate 6.—*A*, Marglobe tomato (*Lycopersicon esculentum*) experimentally infected with celery calico, leaf and leaflet showing light areas on leaflets which were orange in color. *B–H*, Rosy Morn petunia (*Petunia hybrida*) infected with the virus of celery calico from delphinium: *B*, healthy leaf from check or control plant; *C*, leaf showing cleared venation 6 days after inoculation; *D*, *E*, oak-leaf pattern extending along the midrib and lateral veins; *F*, variable orange and green patterns on a lower or basal leaf; *G*, orange discoloration along the veins near the tip of a basal leaf; *H*, orange discoloration near the basal region of a lower leaf and extending along the midrib. *I*, Fern leaf, filiform leaf, or shoestring leaf on Marglobe tomato, sometimes produced by tomato mosaic (identical with ordinary tobacco-mosaic virus, tobacco mosaic 1); but the celery-calico virus separated from tobacco mosaic in a virus complex in naturally infected tomato plants rarely induces such symptoms. *J–K*, Papilio viola (*Viola cornuta*): *J*, flower from a healthy plant used as a check or control plant; *K*, breaking in color of flower from a plant inoculated with the virus extract from delphinium naturally infected with celery calico.

PERENNIAL-DELPHINIUM RINGSPOT

HENRY H. P. SEVERIN AND R. C. DICKSON

PERENNIAL-DELPHINIUM RINGSPOT[1]

HENRY H. P. SEVERIN[2] AND R. C. DICKSON[3]

INTRODUCTION

Among the virus diseases encountered in the course of the investigation of celery calico on perennial delphiniums (*5*)[4] was one which caused ringspot of perennial delphinium; this was found on unknown varieties or hybrids at Berkeley, Hillsborough, and Montara, California, the symptoms being identical in the three places. The symptoms of celery calico on delphinium are confined to the basal or intermediate leaves, whereas ringspots occur on all of the leaves.

Work was undertaken on the host range, properties of the virus, and determination of the vectors. The symptoms of the disease were compared with those of ringspot previously reported on delphiniums.

Valleau (*7*) found a virosis of delphinium in Kentucky closely resembling ringspot of tobacco. He later (*8*) reported a delphinium virus causing a "coarse etch" when transferred to tobacco and suggested seed transmission. In another paper (*9*), he described the symptoms in more detail and gave delphinium, tobacco, tomato, and cucumber as host plants of the virus. The symptoms on delphinium consist of chlorotic ring patterns on individual lobes of the affected leaf, sometimes extending into every lobe. He also stated that this disease occurred naturally in tobacco both in Kentucky and in Minnesota and that "this virus corresponds most closely to the typical cucumber viruses."

Johnson (*3*) found patterns on delphinium similar to the large, yellowish, concentric patterns on dark tobacco caused by a tobacco-ringspot virus identical with that described by Fromme, Wingard, and Priode (*2*).

Burnett (*1*) described a virus disease causing dark-brown to black lesions on delphinium leaves. Tobacco plants inoculated from these showed an irregular mottle with ring-and-line patterns. Delphinium plants inoculated from these tobacco plants developed dark-brown ring-and-line patterns during the first season.

MATERIALS AND METHODS

Source of Virus.—The source of ringspot virus used in the work on host range was delphiniums collected at Montara and Berkeley and that of the virus used in the property studies was a single naturally infected

[1] Received for publication July 17, 1941.

[2] Associate Entomologist in the Experiment Station.

[3] Associate in the Experiment Station.

[4] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

delphinium plant obtained in Berkeley. The virus was retained by repeated mechanical inoculation of susceptible host plants.

Virus Extract.—In preparing juice from infected plants, leaves were ground in a sterilized food chopper or in a mortar. The pulp was then placed in two layers of cheesecloth and the juice pressed out by hand.

Mechanical Inoculation.—The carborundum method of mechanical inoculation used was that described by Rawlins and Tompkins (*4*). Shortly after inoculation the carborundum and inoculum were washed from the leaves with water. The virus extract was usually inoculated into lots of 5 healthy plants. Inoculated plants were observed daily for symptoms. If symptoms failed to develop, tobacco plants were held for 21 days, cucumber plants for 30 days, and plants used in the host-range study for 30 to 60 days or longer, before they were discarded.

Recovery of Virus.—Whether or not symptoms appeared, an attempt was made to recover and transfer the virus from each plant or group of 5 plants used in the host-range studies, to lots of 5 healthy Turkish tobacco or White Spine cucumber plants. The inoculum was taken from young leaves and in some cases from the inoculated leaves also.

Noninfective Insects.—Most of the insects tested to determine the vector of the ringspot virus were from virus-free colonies maintained on caged celery plants in the greenhouse, although a few species of insects were collected in the field. Most of the aphids tested for virus transmission were confined in leaf cages clipped to the leaves of diseased plants and after a feeding period of about 18 hours were shaken from the cages and transferred to healthy seedlings by means of a moist camel's-hair brush. Leafhoppers were captured by means of a 10-cc pipette and were fed on diseased and healthy plants either in leaf cages or in large cages enclosing the plant. Mites were transferred from diseased to healthy plants with a moist camel's-hair brush.

HOST RANGE

The natural host range of delphinium ringspot so far determined is limited to perennial delphiniums. The host range of delphinium ringspot as determined by experimental infection by mechanical inoculation includes 11 species, in 8 genera, in 5 families. Some of the infected host plants developed local lesions, in others the infection was systemic, and one symptomless carrier was demonstrated.

RANUNCULACEAE, CROWFOOT FAMILY

Perennial Delphinium.—The symptoms on the younger leaves of naturally infected perennial delphiniums are faint chlorotic rings, frequently irregular in shape, 1 to 5 mm in diameter, enclosing green (plate

1, *A*) or yellow centers with concentric chlorotic and green lines (plate 1, *A*). Small, irregular, chlorotic areas are scattered between the rings (plate 1, *A*). The mature leaves show irregular chlorotic rings frequently 10 mm in diameter enclosing green areas, yellow bands 1 to 2 mm in diameter, and irregular chlorotic areas 1 to 4 mm wide (plate 1, *B*). Numerous small, concentric, chlorotic rings occur near the margin and in the serrations of the mature leaves (plate 1, *B*). The older leaves show large circular or irregular chlorotic areas surrounded by green and yellow lines (plate 1, *C, D*). Numerous faint chlorotic rings enclosing green centers or masses of small, yellow, circular areas cover more than half of the leaf surface (plate 1, *C*). The first symptoms on the inoculated leaves of Blackmore and Langdon delphinium are pale-green areas, which soon develop into zonate, pale-yellow ringspots 3 to 10 mm in diameter (plate 1, *E*). The first symptoms appeared 32 to 42 days after inoculation.

The virus was easily recovered from naturally infected delphiniums and transmitted to other susceptible hosts by mechanical inoculation.

Turban and Persian Buttercups.—Turban and Persian buttercups (*Ranunculus asiaticus*) were symptomless carriers of the delphinium-ringspot virus. Systemic infection occurred and the virus was easily recovered.

CHENOPODIACEAE, GOOSEFOOT OR SALTBUSH FAMILY

Sugar Beet.—Dark-brown necrotic rings, 0.5 to 1.0 mm in diameter, surrounded by semichlorotic areas 2.0 to 3.0 mm in diameter, sometimes encircled by a broken necrotic ring (plate 2, *A*) developed on the inoculated leaves of sugar beet (*Beta vulgaris*) 21 to 35 days after inoculation. Attempts to recover the virus from beets by juice inoculation to Turkish tobacco and White Spine cucumber plants were unsuccessful.

MALVACEAE, MALLOW FAMILY

Acala Cotton.—No symptoms of the disease developed on the inoculated leaves of Acala cotton (*Gossypium hirsutum*), but dark-brown, irregular, necrotic lesions, 5 mm or less in diameter, sometimes with pale centers, appeared on the young leaves 10 to 12 days after inoculation.

The virus was recovered from this host and transmitted to tobacco and cucumber plants.

SOLANACEAE, NIGHTSHADE FAMILY

Tobacco.—The symptoms of delphinium ringspot on Turkish tobacco (*Nicotiana Tabacum*) consist of zonate, necrotic lesions. The first symptoms to appear on the inoculated leaves 3 to 8 days after inoculation are

brown, shining, sunken, necrotic spots, circular or sometimes irregular in shape and 0.3 to 1.0 mm in diameter (plate 3, *A*). The lesions slowly enlarge during the next day or two (plate 3, *B*), and some of them become surrounded by pale-green halos. Narrow, broken, necrotic rings 3 to 4 mm in diameter appear around each spot 6 to 11 days after inoculation. These broken rings become entire within 1 day and form a green ring about 1 mm wide and the included tissue becomes necrotic (plate 3, *B*). The rings enlarge and become necrotic (plate 3, *C*). When the original spots are less than 2 mm apart, the rings coalesce (plate 3, *D*). A few necrotic rings about 3 mm in diameter may appear in previously normal areas of the leaf. The area surrounded by each ring becomes necrotic within 2 days and may enlarge to become slightly irregular in outline and have a diameter of 6 mm.

The recovery of the virus from inoculated Turkish tobacco plants was possible only while the lesions were still developing. Lesions and small rings of tissue surrounding them were cut from Turkish tobacco leaves 5 days after inoculation; the juice was extracted and inoculated into healthy Turkish tobacco. A few infections were obtained in this manner. The lesions were fully developed 10 days after inoculation, and all attempts to recover the virus from them or from other parts of the plant were unsuccessful. Repeated attempts to recover the virus from inoculated Turkish tobacco plants during a period of 5½ months after their inoculation were failures.

It appears that the virus spreads but a short distance from the point of entrance in this plant, and is rendered inactive by the dying and drying of the tissue that it has invaded.

The symptoms of the disease on White Burley tobacco (*Nicotiana Tabacum*) are similar to those described on Turkish tobacco, and the incubation period is the same, but the necrotic rings are somewhat larger. Attempts to recover the virus from this host plant were unsuccessful.

Nicotiana Glutinosa.—In the early stage of the disease, faint, chlorotic rings enclosing green areas (plate 3, *E*) appear on the inoculated leaves of *Nicotiana glutinosa;* later the peripheries of the rings become necrotic (plate 3, *E*). In the late stage of the disease, concentric rings appeared, followed by complete necrosis within the rings (plate 3, *F*). The lesions become dry and brittle and sometimes are broken and fall out. The incubation period of the disease varied from 15 to 18 days. No systemic infection occurred, and attempts to recover the virus were unsuccessful.

Jasmine Tobacco.—Symptoms on the inoculated leaves of jasmine tobacco (*Nicotiana alata* var. *grandiflora*) are similar to those described for Turkish tobacco. In addition, many of the inoculated leaves develop

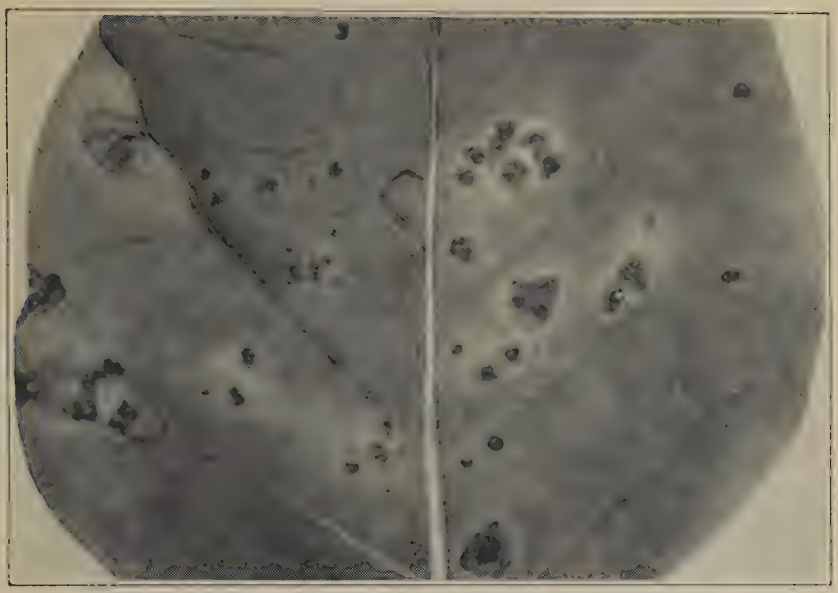

Fig. 1.—*Nicotiana alata* var. *grandiflora:* leaf 40 days after inoculation with delphinium-ringspot virus, showing groups of dark-brown, necrotic lesions and large zonate ringspots.

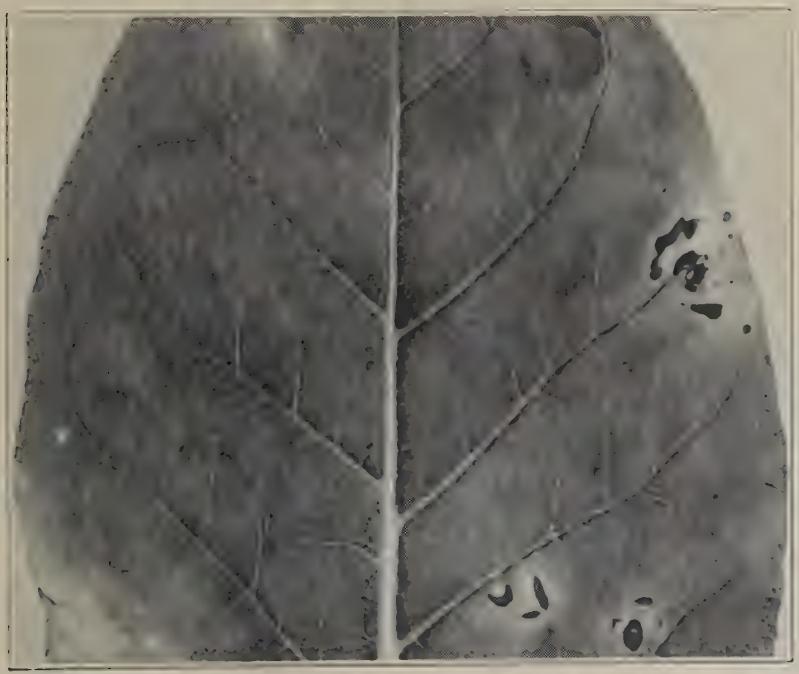

Fig. 2.—*Nicotiana alata* var. *grandiflora:* leaf which developed after inoculation, showing necrotic ringspots surrounded by broken, necrotic rings 10 days after the plant was inoculated with delphinium-ringspot virus.

Fig. 3.—Peasant's tobacco (*Nicotiana rustica* var. *humulis*) : leaf 9 days after inoculation with delphinium-ringspot virus, showing necrotic rings surrounding necrotic areas.

Fig. 4.—Peasant's tobacco (*Nicotiana rustica* var. *humulis*) : leaf 11 days after inoculation with delphinium-ringspot virus, showing small target pattern with necrotic center surrounded by alternating green and necrotic rings.

semichlorotic areas surrounding the necrotic lesions. The infection becomes systemic in this host plant, and leaves appearing after inoculation may develop dark-brown, zonate, necrotic lesions (fig. 1) 30 to 60 days after the plant is inoculated. These spots may be surrounded by partial rings of necrotic tissue (fig. 2). Attempts to recover the virus from this host and transmit it to Turkish tobacco and cucumber were unsuccessful.

Peasant's Tobacco.—The early symptoms of the disease on peasant's tobacco (*Nicotiana rustica* var. *humilis*) are similar to those described on Turkish tobacco. Each necrotic lesion or minute ring becomes surrounded by a necrotic ring which also encloses a ring of green tissue (fig. 3). The ringspot may show a small, target pattern with necrotic center surrounded by alternating green and necrotic rings (fig. 4). Later, the entire area within each ring becomes necrotic.

The incubation period of the disease is 5 to 7 days. No recovery was made.

Petunia.—The early symptoms of ringspot on the leaves of Crimson King petunia (*Petunia hybrida*) 5 to 8 days after inoculation are dark-brown, necrotic lesions 1 mm or less in diameter. These enlarge to attain a diameter of 10 mm 2 weeks after inoculation (plate 4, *A, B, C*). Ten to 14 days after inoculation, necrotic lesions (plate 4, *D*), or necrotic spots (plate 4, *E*) or streaks, sometimes along the veins and midrib (plate 4, *F, G*), or rings appear on the young leaves. These symptoms increase in severity so that 30 days after inoculation the leaves are marked by small necrotic lesions or rings (plate 4, *H*), or necrotic concentric rings (plate 4, *I*), or chlorotic rings enclosing brown, dead tissue, or concentric rings (plate 4, *J*), or irregular masses or bands of necrotic tissue (plate 4, *K, L*).

The virus was easily recovered from infected petunia plants and transferred to healthy Turkish tobacco and cucumber plants.

Jimsonweed.—The symptoms on jimsonweed (*Datura Stramonium*) are necrotic, local lesions (plate 2, *B*) similar to those described on Turkish tobacco, but slightly smaller. The development sequence and incubation period are the same. Inoculated leaves which develop many lesions may abscise prematurely. No systemic infection occurs.

CUCURBITACEAE, GOURD FAMILY

Cucumber.—In White Spine cucumber (*Cucumis sativus*), the first symptoms of ringspot which develop on the inoculated cotyledons and true leaves of seedlings, 5 to 10 days after inoculation, are pale-green, circular areas with indistinct margins and each with a white pin-point center, probably marking the point of entrance of the virus. The circu-

TABLE 1

Plants Unsusceptible to Delphinium-Ringspot Virus

Family and common name	Scientific name	Plants inoculated
		number
Chenopodiaceae, goosefoot or saltbush family:		
Virginia Savoy spinach	*Spinacia oleracea* L.	5
Ranunculaceae, buttercup family:		
Annual larkspur	*Delphinium Ajacis* L.	5
Cardinal larkspur	*Delphinium cardinale* Hook	5
Love-in-a-mist	*Nigella damascena* L.	5
Summer Adonis	*Adonis aestivalis* L.	5
California buttercup	*Ranunculus californicus* Benth.	15
Poppy anemone	*Anemone coronaria* L.	5
Cruciferae, mustard family:		
February cauliflower	*Brassica oleracea* L. var. *botrytis* L.	5
Annual stock	*Mathiola incana* R. Br. var. *annua* Voss	3
Violaceae, violet family:		
King of the Black pansy	*Viola tricolor* L. var. *hortensis* DC.	5
Tropaeolaceae, Tropaeolum family:		
Golden Gleam nasturtium	*Tropaeolum majus* L.	10
Leguminosae, pea family:		
Brabham cowpea	*Vigna sinensis* Endl.	5
Blackeye cowpea	*Vigna sinensis* Endl.	15
Chilean alfalfa	*Medicago sativa* L.	5
Horse bean	*Vicia faba* L.	5
A. & M. Wonder garden pea	*Pisum sativum* L.	5
Lupine	*Lupinus polyphyllus* Lindl.	5
Cucurbitaceae, gourd family:		
Cantaloupe	*Cucumis Melo* L. var. *cantalupensis* Naudin	10
Sugar pumpkin	*Cucurbita Pepo* L.	5
Zucchini squash	*Cucurbita Pepo* L.	10
White Bush Scallop squash	*Cucurbita Pepo* L.	5
Umbelliferae, parsley family:		
Golden Self-blanching celery	*Apium graveolens* L. var. *dulce* DC.	15
Blue laceflower	*Trachymene caerulea* R. Graham	5
Labiatae, mint family:		
Scarlet sage	*Salvia splendens* Ker	3
Solanaceae, nightshade family:		
Seedling potato	*Solanum tuberosum* L.	2
Black Beauty eggplant	*Solanum Melongena* L.	11
Marglobe tomato	*Lycopersicon esculentum* Mill.	58
California Wonder bell pepper	*Capsicum frutescens* L.	5
Scrophulariaceae, figwort family:		
Snapdragon	*Antirrhinum majus* L.	5
Compositae, sunflower family:		
Large Russian sunflower	*Helianthus annuus* L.	5
China aster	*Callistephus chinensis* Nees	7
Romaine lettuce	*Lactuca sativa* L. var. *longifolia* Lam.	8

lar areas become bright yellow in color and vary from 4 to 6 mm in diameter (plate 2, *C*). An indistinct chlorotic area may later extend from each of the circular spots to adjacent parts of the leaf, sometimes followed by necrosis. After the infection becomes systemic, 9 to 18 days after inoculation, numerous chlorotic rings enclosing green centers or

necrotic spots and chlorotic circular areas appear on the leaves (plate 2, *D*).

The interspaces between the veinlets are sometimes filled by small chlorotic areas (plate 2, *E*), and these may coalesce, especially along the margin of the leaf (plate 2, *F*). Frequently the leaves become chlorotic

TABLE 2

THERMAL INACTIVATION OF DELPHINIUM-RINGSPOT VIRUS

Plants inoculated, source of virus, and no. of source plant	Plants infected of 5 inoculated							
	Unheated control	45° C	50° C	55° C	60° C	65° C	70° C	75° C
	number	*number*	*number*	*number*	*number*	*number*	*number*	*number*
From delphinium into Turkish tobacco:								
1	5	5	5	1	1	0	0	0
2	5	5	3	0	0	0	0	0
From cucumber into Turkish tobacco:								
3	5	5	3	2	0	0	0	0
4	5	5	5	0	0	0	0	0
5	5	3	1	0	0	0	0	0
6	5	5	5	0	0	0	0	0
7	5	5	1	0	0	0	0	0
8	2	2	4	0	0	0	0	0
From cucumber into cucumber:								
6	5	5	4	0	0	0	0	0
7	5	5	4	0	0	0	0	0
8	2	5	4	1	0	0	0	0
Total, all sources	49	50	39	4	1	0	0	0
Percentage	*89.1*	*90.9*	*70.9*	*7.3*	*1.8*	*0.0*	*0.0*	*0.0*

with green vein-banding (plate 2, *F*), or may become almost entirely chlorotic; they are usually recurved. The plants become brittle and stunted and are often killed under greenhouse conditions.

This virus is easily recovered from cucumber plants and transmitted to Turkish tobacco and cucumber plants by juice inoculation.

PLANTS UNSUSCEPTIBLE

No infection was obtained by mechanical inoculation in 28 species of plants representing 26 genera in 12 families as shown in table 1. Attempts were made to recover the virus from each lot of 5 plants.

PROPERTIES OF VIRUS

Thermal Inactivation.—The thermal inactivation of the virus was determined with undiluted, extracted juices from the leaves of a naturally infected delphinium plant and also from the leaves and stems of

TABLE 3

TOLERANCE TO AGING IN VITRO OF DELPHINIUM-RINGSPOT VIRUS AT ROOM TEMPERATURE

Plants inoculated, source of virus, and no. of source plant	Plants infected of 5 inoculated after various periods of aging															
	Control	4 hours	8 hours	12 hours	18 hours	1 day	1½ days	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days
	number	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*
From delphinium into Turkish tobacco:																
1	5	5	5	5	2	0	0	0	0	0	0	0	0	0	0	0
From cucumber into Turkish tobacco:																
2	5	5	5	3	4	4	5	5	3	1	0	0	0	0	0	0
3	5	5	5	5	2	4	2	4	2	0	0	0	0	0	0	0
4	5	5	5	5	3	4	4	0	0	0	0	0	0	0	0	0
5	4	3	4	1	1	3	1	0	0	0	0	0	0	0	0	0
6	4	2	4	1	4	3	2	0	0	0	0	0	0	0	0	0
7	5	5	5	5	5	2	0	0	0	0	0	0	0	0	0	0
From cucumber into cucumber:																
2	4	3	5	3	3	3	5	2	3	1	0	0	0	0	0	0
3	4	3	1	2	0	1	3	3	2	0	0	0	0	0	0	0
4	1	4	3	4	4	5	2	0	1	2	0	0	0	0	0	0
Total, all sources	42	40	42	34	28	29	24	14	11	4	0	0	0	0	0	0
Percentage	*84.0*	*80.0*	*84.0*	*68.0*	*56.0*	*58.0*	*48.0*	*28.0*	*22.0*	*8.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*

TABLE 4

TOLERANCE TO DILUTION OF DELPHINIUM-RINGSPOT VIRUS

Plants inoculated, source of virus, and no. of source plant	Plants infected of 5 inoculated, after various dilutions									
	Undiluted control	1:10	1:100	1:500	1:1,000	1:2,000	1:5,000	1:10,000	1:50,000	1:100,000
	number	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*	*number*
From delphinium into Turkish Tobacco:										
1	5	5	5	5	2	0	0	0	0	0
2	5	5	5	3	1	0	0	0	0	0
3	5	5	3	2	0	0	0	0	0	0
4	4	5	3	2	0	0	0	0	0	0
From cucumber into Turkish tobacco:										
5	5	5	3	1	1	0	0	0	0	0
6	5	5	4	1	0	0	0	0	0	0
7	5	5	2	0	0	0	0	0	0	0
8	5	4	2	0	0	0	0	0	0	0
9	5	2	1	0	0	0	0	0	0	0
10	4	5	2	0	0	0	0	0	0	0
From cucumber into cucumber:										
5	5	5	3	0	0	0	0	0	0	0
6	5	5	4	1	0	0	0	0	0	0
10	4	5	0	2	0	0	0	0	0	0
Total	62	61	37	17	4	0	0	0	0	0
Percentage	*95.4*	*93.8*	*56.9*	*26.1*	*6.2*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*

experimentally infected cucumber plants. Ten cc of juice from diseased plants was placed in each sterile, thin-walled, test tube by means of a pipette to avoid contamination of the lip of the tube. Each test tube containing the virus extract was immersed in the water bath at the desired temperature for 11 minutes, about 1 minute being required for the heat to penetrate the glass of the test tube. The bath was maintained within 0.5 degree of the desired temperature throughout each test. The water was kept in circulation by an agitator connected to an electric motor. After exposure to the desired temperature, the test tubes were cooled rapidly in running water. After cooling, the juice was poured into a small culture dish and the plants were inoculated without delay. Unheated controls were used in each test. The results obtained are indicated in table 2. The delphinium-ringspot virus was active after heating the expressed juice from delphinium and cucumber plants at 45°, 50°, and 55° C. A single infection was obtained after heating the virus extract from delphinium at 60°. The virus was inactivated by heating to 65°.

Tolerance to Aging in Vitro.—To determine the resistance of the ringspot virus to aging in vitro, test tubes each containing 10 cc of expressed juice from diseased delphinium or cucumber plants were plugged with cotton and kept in the dark at room temperature. Fresh-extract controls were used in each trial. The juice was tested for infectivity by mechanical inoculation after periods of 4, 8, 12, and 18 hours, 1 day, 1½ days, and then daily until 10 days had elapsed. The results are shown in table 3.

It is evident that the virus was active in the cucumber extract in vitro for various periods up to and including 4 days, but was inactivated after 5 days. One virus extraction from delphinium resulted in 2 infections after 18 hours' aging in vitro, but none after 1 day.

Tolerance to Dilution.—The tolerance to dilution of the ringspot virus was determined by diluting the expressed juice from diseased delphinium and cucumber plants with distilled water. The higher dilutions were inoculated first to minimize the danger of accidental infection. Undiluted controls were used in each test. Table 4 indicates the results obtained.

Infections were occasionally obtained at a dilution of 1:1,000 with the extract from delphinium and cucumber plants but not at 1:2,000 or at higher dilutions.

ATTEMPTS TO DETERMINE THE VECTOR

A limited number of insects and mites occur on delphinium under natural conditions in California. The mountain leafhopper (*Thamnotettix montanus* Van D.), the geminate leafhopper (*Thamnotettix gemi-*

TABLE 5

INSECTS AND MITE THAT FAILED TO TRANSMIT RINGSPOT VIRUS

Common and scientific name of insects and mite	Insect or mite			Plants used
	Average number on each plant	Period on diseased plant	Period on healthy plant	
From diseased to healthy delphinium				
	number		*days*	*number*
Aster leafhopper, *Macrosteles divisus* (Uhl.)	15	12 hours	3	10
Geminate leafhopper, *Thamnotettix geminatus* Van D.	24	5 days	24	10
Mountain leafhopper, *Thamnotettix montanus* Van D.	22	6 days	17	10
Celery leaf aphid, *Aphis apigraveolens* Essig	27	18 hours	2	10
Celery aphid, *Aphis apii* Theob.	33	18 hours	2	10
Cotton, or melon, aphid, *Aphis gossypii* Glover	23	18 hours	4	10
Erigeron root aphid, *Aphis middletonii* Thomas	29	18 hours	4	10
Cabbage aphid, *Brevicoryne brassicae* (Linn.)	30	18 hours	3	10
Yellow willow aphid, *Cavariella capreae* (Fabr.)	23	18 hours	2	10
Turnip, or false cabbage, aphid, *Lipaphis pseudobrassicae* (Davis)	29	18 hours	2	10
Onion aphid, *Micromyzus formosanus* Taka	31	18 hours	3	10
Lily aphid, *Myzus circumflexus* Buckton	28	18 hours	6	10
Foxglove aphid, *Myzus convolvuli* (Kalt.)	30	18 hours	8	10
Green peach aphid, *Myzus persicae* (Sulz.)	25	18 hours	7	25
Honeysuckle aphid, *Rhopalosiphum melliferum* (Hottes)	28	18 hours	3	10
Rusty-banded aphid, *Aphis ferruginea-striata* Essig	29	18 hours	2	10
Two-spotted mite, *Tetranychus bimaculatus* Harvey	25	Reared	7	10
From diseased to healthy cucumber plants				
	number	*days*	*days*	*number*
Agallia californica (Baker)	7	2	5	1
Blue-green sharpshooter, *Cicadella circellata* (Baker)	12	2	5	2
Western potato leafhopper, *Empoasca abrupta* De L.	5	2	5	2
Cotton or melon aphid, *Aphis gossypii* Glover	23	2–5	2	20
Erigeron root aphid, *Aphis middletonii* Thomas	20	2	2	10
Cabbage aphid, *Brevicoryne brassicae* (Linn.)	15	4	2	1
Turnip, or false cabbage, aphid, *Lipaphis pseudobrassicae* (Davis)	15	4	2	5
Onion aphid, *Micromyzus formosanus* Taka	8	4	2	2
Lily aphid, *Myzus circumflexus* Buckton	24	2	2	22
Green peach aphid, *Myzus persicae* (Sulz.)	24	2	3	20
Rusty-banded aphid, *Aphis ferruginea-striata* Essig	20	2	2	10
Two-spotted mite, *Tetranychus bimaculatus* Harvey	22	Reared	4	10
From diseased to healthy turban and Persian buttercup plants				
	number		*days*	*number*
Ornate aphid, *Myzus ornatus* Laing	17	Reared	10	15
Cyclamen mite, *Tarsonemus pallidus* Banks	10	Reared	30	10

natus Van D.) and two species of mites—the two-spotted mite (*Tetranychus bimaculatus* Harvey), and the cyclamen mite (*Tarsonemus pallidus* Banks)—were found breeding on delphinium. An occasional green peach aphid (*Myzus persicae* [Sulz.]) and undetermined dead winged aphids were found on this plant under natural conditions. The insects and the mite which failed to transmit the ringspot virus from diseased to healthy delphinium are listed in table 5.

Since the virus is easily transmitted to various host plants by mechanical inoculation, it was assumed that the vector is probably an aphid. Tests were made with colonies of various species of aphids maintained in the greenhouse. Insects which could not be reared on delphinium were fed on diseased plants for periods of from 12 to 18 hours and then were transferred to healthy delphinium seedlings.

Daily observations were made on the mortality of the insects, and after the last insect of a lot was dead (table 5), the seedlings were fumigated. The last living specimen of an average lot of 30 foxglove aphids (*Myzus convolvuli* [Kalt.]) survived on seedling delphinium for 8 days, green peach aphid, (*M. persicae* [Sulz.]) for 7 days, lily aphid (*M. circumflexus* Buckton) for 6 days, and all other species of aphids for 2 to 4 days, as shown in table 5.

No transmission of the virus was obtained with any of the various species of aphids tested.

Unsuccessful attempts were made to transmit the virus by means of leafhoppers, aphids, and mites from White Spine cucumbers infected with the ringspot virus to healthy cucumber plants. A list of insects and mites that failed to transmit the ringspot virus from diseased to healthy cucumber plants is given in table 5.

Transmission of the virus from diseased to healthy turban and Persian buttercups (*Ranunculus asiaticus*) was attempted, with aphids and mites but was unsuccessful. A list of the aphids and mites which failed to transmit the virus is shown in table 5.

DISCUSSION

Valleau (*9*) lists delphinium, Turkish tobacco, tomato, and cucumber as hosts of a virus found in delphinium in Kentucky. Tomato plants could not be infected with the California delphinium-ringspot virus. A total of 58 Marglobe tomato plants were inoculated, but no symptoms developed, and all attempts to recover the virus from these plants were failures.

The symptoms described by Valleau (*9*) on delphinium and Turkish tobacco as produced by the virosis on delphinium in Kentucky are not identical with the symptoms produced on the same host plants by the

ringspot virus in California. It is therefore evident that the two viruses are not identical.

The symptoms of tobacco ringspot on delphinium described by Johnson (*3*) differed from those produced on this host plant by the delphinium-ringspot virus in California. The symptoms of tobacco ringspot on delphinium and cucumber are described in another paper of this series (*6*).

The symptoms described by Burnett (*1*) on delphinium and tobacco also differed from those produced on these host plants by the delphinium-ringspot virus in California.

DESCRIPTION OF DELPHINIUM-RINGSPOT VIRUS

Name: Delphinium ringspot.

Host families: Ranunculaceae, Chenopodiaceae, Malvaceae, Solanaceae, and Cucurbitaceae.

Symptoms of disease: On young leaves of delphinium faint chlorotic rings enclosing green or yellow centers; on mature leaves, irregular chlorotic rings encircling green areas, yellow bands, and irregular chlorotic areas.

Incubation period of disease: 32 to 42 days in the greenhouse.

Property studies: Thermal inactivation 65° C in 10 minutes' exposure, tolerance to dilution 1:1,000, and resistance to aging in vitro 5 days.

Modes of transmission: Mechanical inoculation with expressed juice, in nature vector was not found.

SUMMARY

Perennial or garden delphinium was demonstrated to be naturally infected with an undescribed ringspot virus.

The host range of the ringspot virus as determined by mechanical inoculation includes 11 species of plants in 8 genera belonging to 5 families, as follows:

Ranunculaceae, crowfoot family:
- Blackmore and Langdon perennial delphinium (*Delphinium* sp.), systemic infection, virus recovered.
- Turban and Persian buttercups (*Ranunculus asiaticus*), symptomless carrier, virus recovered.

Chenopodiaceae, goosefoot or saltbush family:
- Sugar beet (*Beta vulgaris*), local infection, virus not recovered.

Malvaceae, mallow family:
- Acala cotton (*Gossypium hirsutum*), systemic infection, virus recovered.

Solanaceae, nightshade family:
- Turkish tobacco (*Nicotiana Tabacum*), local infection, virus recovered only while the lesions were developing.
- White Burley tobacco (*Nicotiana Tabacum*), local infection, virus not recovered.
- *Nicotiana glutinosa*, local infection, virus not recovered.
- *Nicotiana alata* var. *grandiflora*, systemic infection, virus not recovered.
- Peasant's tobacco (*N. rustica* var. *humulis*), local infection, virus not recovered.

Crimson King petunia (*Petunia hybrida*), systemic infection, virus recovered.
Jimsonweed (*Datura Stramonium*), local infection, virus not recovered.
Cucurbitaceae, gourd family:
White Spine cucumber (*Cucumis sativus*), systemic infection, virus recovered.

Twenty-eight species of plants in 26 genera in 12 families were inoculated with the ringspot virus but proved unsusceptible.

The thermal inactivation of the ringspot virus was 65° C in a 10-minute exposure. Inactivation of the virus occurred after the extracted juice from diseased cucumber plants was exposed to the air at room temperature for a period of 5 days. The tolerance to dilution of extracted juice from diseased delphinium and cucumber plants was 1:1,000.

All attempts to find a vector of the ringspot virus were failures.

ACKNOWLEDGMENTS

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PLATES

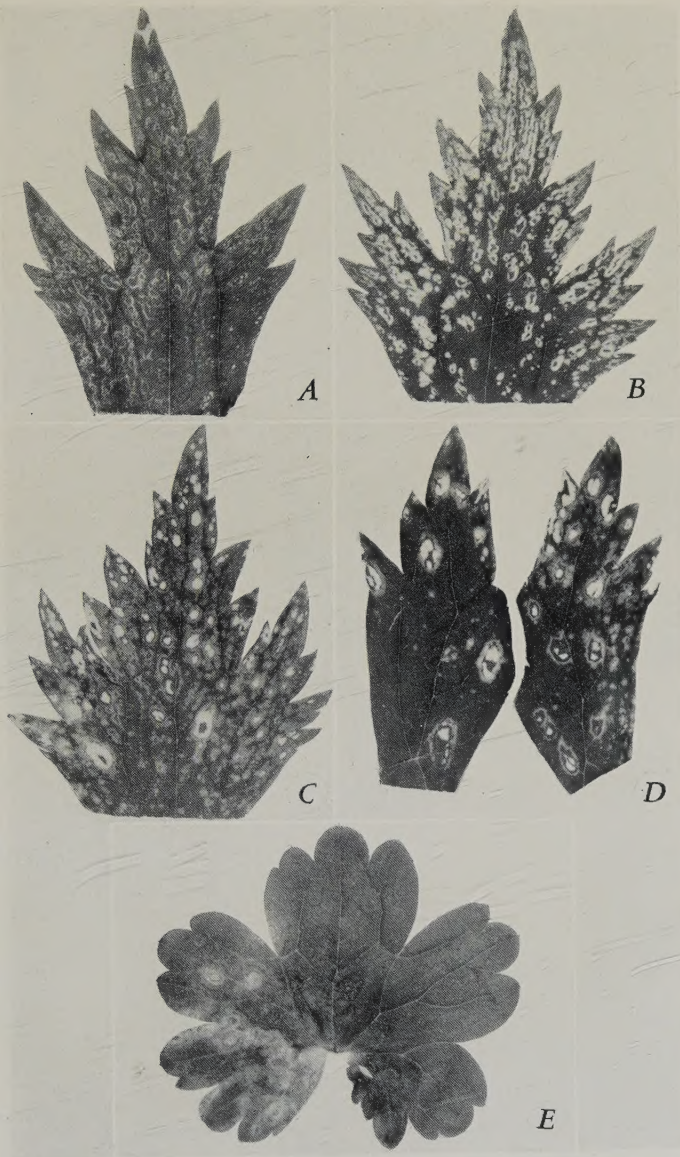

Plate 1.—Perennial delphinium naturally infected with ringspot: *A*, lobe of young leaf showing faint, chlorotic rings enclosing green or yellow centers, and small, irregular, chlorotic areas scattered between the rings; *B*, lobe of mature leaf from the same plant showing irregular, chlorotic rings enclosing green areas, yellow bands, irregular, chlorotic areas, and numerous, small, concentric rings near the margin and in the serrations; *C*, lobe of old leaf from the same plant showing large, circular or irregular, chlorotic areas, each surrounded by a green line, and sometimes with a green center, also masses of small yellow, circular areas, and faint chlorotic rings enclosing green centers; *D*, enlarged lobes of mature leaves from the same plant showing yellow rings enclosing chlorotic or green areas or both; *E*, inoculated leaf from Blackmore and Langdon delphinium showing pale, yellow ringspots with concentric lines.

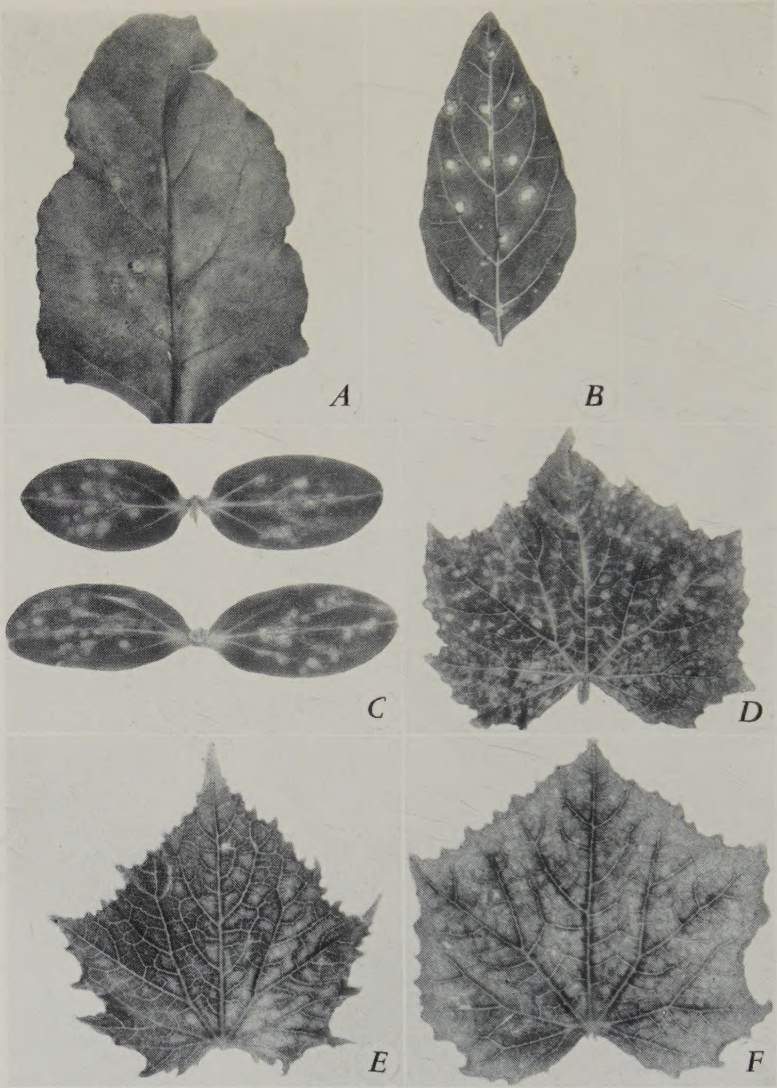

Plate 2.—Symptoms of delphinium ringspot on leaves of various host plants: *A*, leaf of sugar beet (*Beta vulgaris*) showing necrotic rings surrounded by chlorotic areas 24 days after inoculation; *B*, leaf of jimsonweed (*Datura Stramonium*) showing concentric rings 13 days after inoculation; *C*, cotyledons of White Spine cucumber (*Cucumis sativus*) showing circular, yellow areas, each with a white or necrotic pin point in the center, 11 days after inoculation; *D*, leaf of cucumber plant 26 days after inoculation, showing numerous, chlorotic rings enclosing green centers or necrotic spots; *E*, leaf of cucumber plant 23 days after inoculation, showing chlorotic areas in the interspaces of the veinlets; *F*, leaf of cucumber plant showing numerous chlorotic areas which frequently coalesce, and green vein-banding.

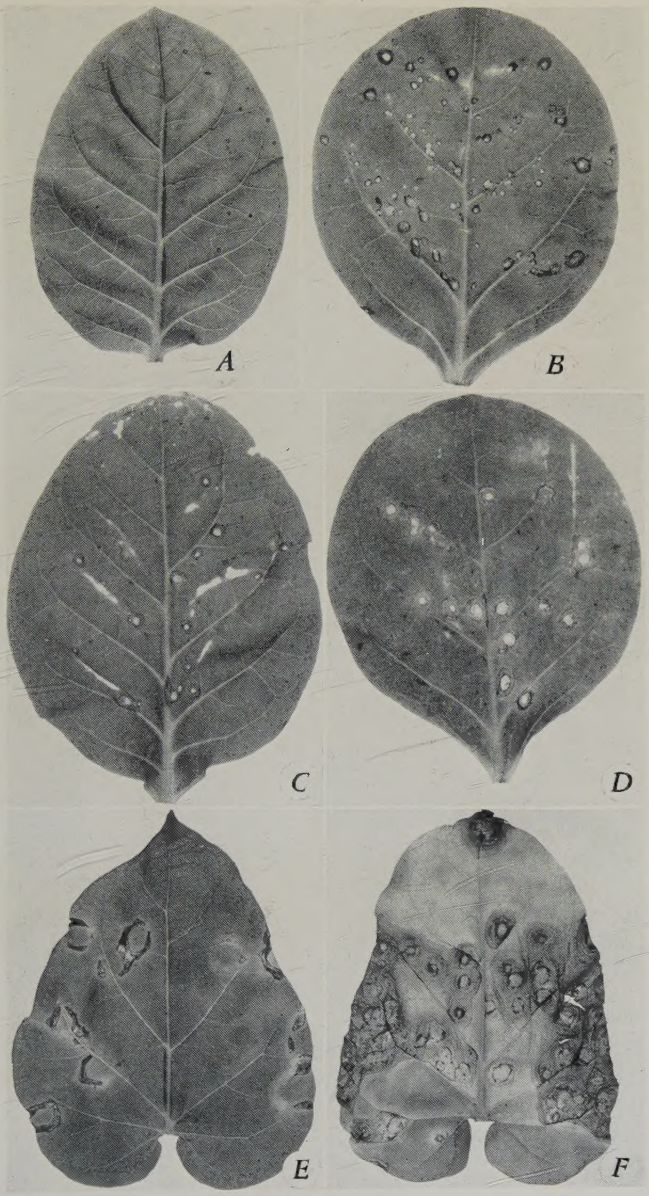

Plate 3.—Symptoms of delphinium ringspot on Turkish tobacco (*Nicotiana Tabacum*) and *N. glutinosa*: *A*, leaf showing small, necrotic lesions 4 days after inoculation; *B*, leaf 6 days after inoculation, showing necrotic areas enlarged and with centers bleached; *C*, leaf 9 days after inoculation, showing necrotic areas surrounded by wider rings—white areas are abrasions caused by inoculation with carborundum; *D*, leaf 10 days after inoculation, showing ringspots, some of which had coalesced; *E*, leaf of *N. glutinosa* showing early stage of ring formation with a faint, wide, chlorotic ring enclosing a green area; later, the periphery of the rings becomes necrotic; *F*, leaf of *N. glutinosa* 4 weeks after inoculation, showing concentric rings and complete necrosis within the rings.

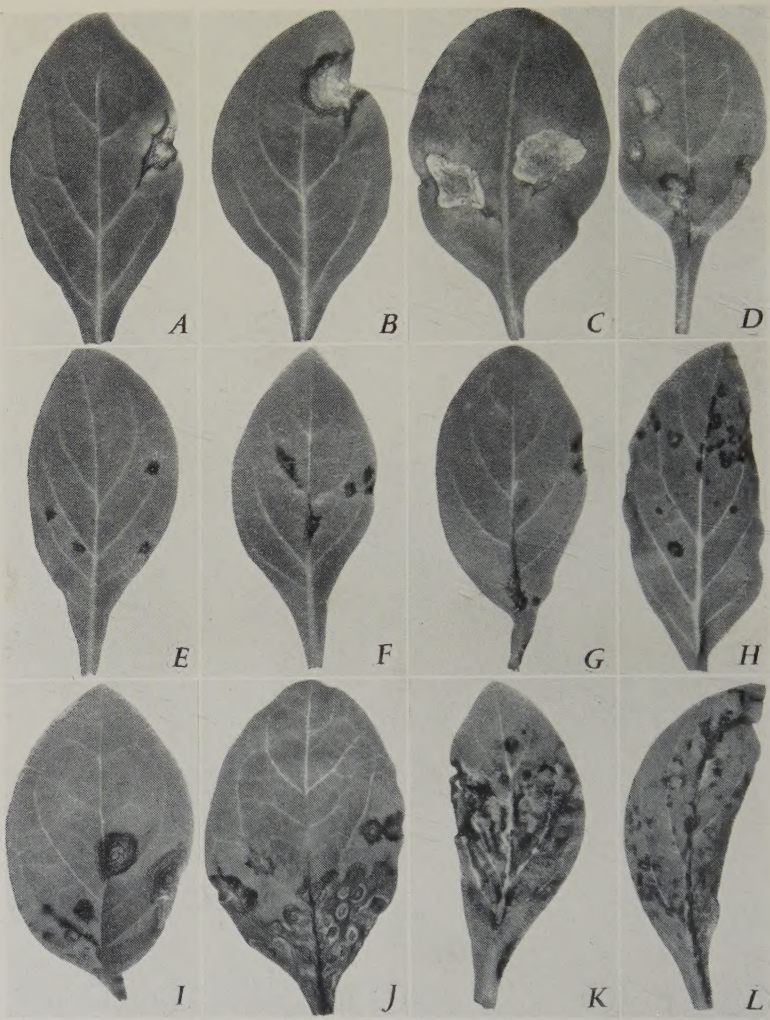

Plate 4.—Crimson King petunia (*Petunia hybrida*) infected with delphinium-ringspot virus: *A*, *B*, *C*, leaves showing large, necrotic lesions 14 days after inoculation; *D*, necrotic lesions on new leaf; *E*, *F*, *G*, necrotic spots or streaks, sometimes along the veins or midrib on young leaves; *H*, small, necrotic rings on leaves which developed 30 days after inoculation; *I*, necrotic, concentric rings; *J*, chlorotic rings enclosing brown, dead tissue; *K*, *L*, irregular masses or bands of necrotic tissue.